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ERRATA

Page 179, table III, second column heading, for "Average," read Total.
Cover page four, April number, sixth title, for "*Photoperiod*" read *Photoperiodic.*
Page 415, author's initial, for "B" read S.
Page 434, in the regression equation, for "1000" read 10000.
Page 435, in both regression equations, for "1000" read 10000.
Cover page four, July number, first title, for "*on distribution*" read *on nitrogen distribution.*
Cover page four, July number, first author's initial, for "B" read S.

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THE PROCESS OF OIL FORMATION AND ACCUMULATION IN THE MACADAMIA[1]

WINSTON W. JONES AND LILY SHAW

In previous investigations of the problem of oil formation in the pecan it has been shown (**4, 16**) that the fatty acids, and consequently the oil and fats, are formed from carbohydrates, either stored or currently synthesized. The present paper deals with formation and changes in characteristics of the oil in the Macadamia from the young embryo to the mature fruit.

The oil present in the mature Macadamia nut (*Macadamia ternifolia* F.v.M. var. *integrifolia* Maiden) is very similar to pecan oil (**2**), consisting largely of the glyceride of oleic acid with small amounts of the glycerides of palmitic, stearic, and myristic acids (**3**). It has a saponification value of 197.3, an acid value of 0.57, and an iodine number of 75.2. As pointed out above, carbohydrates provide the principal carbon source from which fats are formed, but M'CLENAHAN (**9**) believed that in the case of black walnuts the oil was derived from tannins; SCURTI and TOMMASI (**13**) thought that the higher alcohol, oleanol, was converted into oil in the olive.

The formation of limited amounts of fatty acids from hexoses outside of the living cell has been accomplished by SMEDLEY (**14**) who prepared these acids by first breaking down hexoses into C_3 compounds followed by oxidation of the latter into pyruvic acid which was then condensed into acids with longer carbon chains. It is also of interest to note that fatty acids are prepared commercially from the paraffin hydrocarbons (**5**).

Materials and methods

All plant material herein described was collected on June 1, 1940, from an orchard near Honolulu, T. H. The fruiting habits of the Macadamia are such that at this season of the year nuts of all ages from very young to mature can be collected from a single tree. The samples collected from each of six seedling trees were brought to the laboratory and the nuts from each tree divided into five age groups based on known condition of shell as related to age (**7**). Each sample was composed of about one hundred and forty 90-day-old fruits and 35 fruits from each subsequent age group from each

[1] Published with the approval of the Director as Technical Paper no. 100 of the Hawaii Agricultural Experiment Station.

of six trees. Since the carbohydrate and ether extract contents of the Macadamia husk and shell change very little during their development (**7**), these portions of the fruits were discarded and only embryos were studied. A description of the fruits at the time of harvest, including the approximate number of days from flowering for each age group and the average fresh weight per embryo, is given in table I.

TABLE I

CONDITION OF MACADAMIA FRUITS OF DIFFERENT AGE GROUPS AT TIME OF SAMPLING

AGE GROUP	TIME AFTER FLOWERING	AVERAGE FRESH WEIGHT PER EMBRYO	APPEARANCE AND TEXTURE OF SHELL
	days	*gm.*	
1	90	1.14	Soft and white
2	111	2.81	Hard and white
3	136	3.41	Hard and light brown
4	185	2.88	Hard and brown
5	215	2.78	Hard and dark brown

DRY WEIGHT

The youngest samples were dried at 100° C. for 2 hours in an air oven and then in a vacuum oven at 80° C. for 48 hours. Older samples required 8 to 24 hours longer in the air oven adjusted to 80° C. On removal from the vacuum oven the embryos were cooled over calcium chloride and dry weights obtained after which the material was chopped into small particles by means of a sharp knife, redried and returned to the desiccator and held until analyzed.

ANALYTICAL METHODS

OIL.—The redried, finely chopped material was removed from the desiccator and about 2-gram aliquots, the exact weights of which were recorded, were weighed into alundum cups and extracted with anhydrous petroleum ether in a continuous drip extractor for 6 hours. The sample was then removed, reground in an agate mortar, and re-extracted until free of oil. The petroleum ether was removed, and the extract dried, weighed and reported as oil. The oil was then held in the desiccator for the determination of oil characteristics.

SUGARS.—The sugars in the oil-free residue were extracted with 80 per cent. alcohol. An aliquot of the extract was freed of alcohol, cleared with neutral lead acetate and potassium oxalate, and reducing sugars determined by the method of STILES, PETERSON, and FRED (**15**). After treatment with invertase, sucrose was determined.

NITROGEN.—Soluble nitrogen was determined on aliquots of the alcoholic extract by the reduced iron method of PUCHER, LEAVENWORTH, and VICKERY (**11**) adapted to the micro-Kjeldahl method described by PREGL (**10**). Insoluble nitrogen was determined on the residue of the alcoholic extraction by the above micro-method.

ACID-HYDROLYZABLE MATERIALS.—These were obtained from an aliquot of the residue from the alcoholic extraction by determining the amount of reducing substances formed after digestion with 2.5 per cent. HCl under a reflux condenser for three hours.

OIL CHARACTERISTICS.—The ether extract was analyzed according to the official methods of the Association of Official Agricultural Chemists (**1**) with the exception of hydroxyl number determinations which were obtained by the sealed tube method of ROBERTS and SCHUETTE (**12**).

Results and discussion

The data are presented in two parts: (1) those having to do with the accumulation of oil, carbohydrates, and nitrogen; and (2) those having to do with the analysis of the ether extracts.

ACCUMULATION OF OIL, CARBOHYDRATES, AND NITROGEN

The accumulation of dry matter in the Macadamia embryo (table II) is

TABLE II

DRY WEIGHT CHANGES IN THE MACADAMIA FRUIT

AGE GROUP	TIME AFTER FLOWERING	AVERAGE DRY WEIGHT PER EMBRYO	FRESH WEIGHT PER EMBRYO	PERCENTAGE DRY WEIGHT
	days	*gm.*	*gm.*	%
1	90	0.067	1.138	5.0
2	111	0.491	2.806	17.5
3	136	0.992	3.410	29.1
4	185	1.382	2.877	48.0
5	215	1.882	2.781	67.7

continuous from the time of the first sample through to maturity. This is not the case for fresh weight where the peak is attained rather early in the life of the fruit with a consequent slow drying of the embryo so that at the time of maturity the dry material consists of 67.7 per cent. of the total weight of the embryo.

The data on the accumulation of oil, carbohydrates, and nitrogen are shown in tables III and IV. The results presented in tables III and IV agree closely with those already published (**7, 8**); they will be discussed more fully in connection with the formation of oil.

The Macadamia plant, for the first 90 days after flowering, is largely occupied with the laying down of structures of the fruit and the growth of the husk, shell, and endosperm. Later the embryo enlarges rapidly and begins the formation and accumulation of oil. During the period from 90 to 185 days after flowering, 44 per cent. of the total growth period of the fruit, 70 per cent. of the total oil is formed. It is of interest to note that during the accumulation of oil, protein and sucrose also accumulated.

TABLE III

CARBOHYDRATES, NITROGEN, AND OIL CHANGES IN THE MACADAMIA EMBRYO, EXPRESSED AS PERCENTAGE OF DRY WEIGHT, IN RELATION TO AGE

CONSTITUENT	DAYS AFTER FLOWERING				
	90	111	136	185	215
	%	%	%	%	%
Reducing sugar	1.47	3.21	1.07	0.41	0.30
Sucrose	6.07	24.07	21.91	9.19	5.50
Total sugar	7.54	27.28	22.98	9.60	5.80
Soluble nitrogen	2.92	1.13	0.61	0.33	0.27
Insoluble nitrogen	1.96	1.91	1.58	1.39	1.43
Total nitrogen	4.88	3.04	2.19	1.72	1.70
Acid-hydrolyzable matter	4.54	4.88	3.85	2.56	2.16
Soluble solids in 80 per cent. alcohol	60.10	39.92	28.36	14.82	9.88
Ether and alcohol insoluble material	36.43	28.88	23.69	17.89	16.68
Petroleum ether extract	3.46	31.19	47.94	67.28	73.44

ANALYSIS OF ETHER EXTRACT

Table V shows the changes in the oil characteristics during the development of the embryo.

It is clear from table V that the most marked changes in the oil characteristics occur during the 21-day period from 90 to 111 days after flowering. During this period the acid number drops from 163.9 to 6.56 with a final value of 0.57; the saponification number drops from 380.6 to 204.2 with a final value of 197.3; soluble acids drop from 23.65 to 1.67 per cent. with a final value of 0.26 per cent.; and the iodine number increases from 64.4 to 75.4 with a final value of 75.2. The unsaponifiable material is never above one per cent., indicating that the ether extract consists largely of fatty acids or esters. It is apparent that in the early stages of oil formation

TABLE IV

CARBOHYDRATE, NITROGEN, AND OIL CHANGES IN THE MACADAMIA EMBRYO, EXPRESSED AS MILLIGRAMS PER EMBRYO, IN RELATION TO AGE

CONSTITUENT	DAYS AFTER FLOWERING				
	90	111	136	185	215
	mg.	*mg.*	*mg.*	*mg.*	*mg.*
Reducing sugar	0.85	13.69	8.39	5.41	3.97
Sucrose	3.93	115.03	196.65	128.25	100.87
Total sugar	4.78	128.72	205.04	133.66	104.84
Soluble nitrogen	1.94	5.26	5.33	4.33	4.75
Insoluble nitrogen	1.33	8.28	14.35	19.08	26.12
Total nitrogen	3.27	13.54	19.68	23.41	30.87
Acid-hydrolyzable matter	3.00	23.27	34.34	33.43	39.41
Soluble solids in 80 per cent. alcohol	40.02	187.25	251.65	201.20	177.72
Ether and alcohol insoluble material	24.92	139.29	213.54	245.12	304.22
Petroleum ether extract	2.40	164.95	456.81	936.02	1339.39

short-chain fatty acids accumulate as such and these are not esterified until somewhat later. While the accumulation of oil in its final form tends to obscure the actual process of formation the data presented shed some light on the change from carbohydrates to fats.

In the Macadamia it is likely that carbohydrates are the most important source of the oil. Microchemical tests have shown that flowering branches contain considerable quantities of stored starch while non-flowering branches on the same tree often contain no trace of starch. Thus there are available two sources of carbohydrates: stored starch, and that from current photosynthesis. The starch in the flowering branches, however, largely disappears by the time oil formation begins in the developing embryo, which in the Macadamia is the organ of oil formation and storage (**6, 8**). No starch has ever been found in the embryo during development, and oil formation

TABLE V

CHANGES IN THE CHARACTERISTICS OF MACADAMIA OIL IN RELATION TO AGE

CHARACTERISTICS	DAYS AFTER FLOWERING				
	90	111	136	185	215
Acid number	163.90	6.56	2.26	0.73	0.57
Saponification number	380.6	204.2	199.7	197.7	197.3
Soluble acids (per cent.)	23.65	1.67	0.46	0.23	0.26
Insoluble acids (per cent.)	48.14	81.49	86.42	94.09	94.21
Iodine number	64.4	75.4	74.4	75.7	75.2
Unsaponifiable matter (per cent.)	*	0.75	0.58	0.42	0.34
Hydroxyl number	.....	11.1	9.0	6.0	4.8
Index of refraction, 25° C.	.....	1.4681	1.4669	1.4658	1.4657

* Insufficient material for analysis.

necessitates the conversion of the hexoses and/or sucrose that are moved into the embryo into fatty acids and glycerol for the formation of the glycerides.

The movement of materials (soluble carbohydrates and soluble nitrogen) into, and the accumulation of non-mobile materials (oil and protein) in, the Macadamia embryo proceed rapidly as the embryo enlarges. Since the embryo at the onset of rapid accumulation consists of only a few cells, and since the husk and shell change very little in composition (**7**), this material undoubtedly moves into the fruit from outside sources. Aside from the mineral constituents and soluble nitrogen compounds, this material most likely was moved into the embryo as carbohydrates, probably hexose sugars (**4**). If this is the case, the hexose sugars are immediately transformed into other compounds as there is never found any accumulation of reducing sugars.

In a study of the oil characteristics of Macadamia, the accumulation of glycerides obscures the early steps in oil formation since a large percentage of the ether extract consists of these "fully formed" glycerides. Thus the process of oil formation might appear to be somewhat different in the early

stages as compared to the later stages; this is not necessarily true, however, since the process may be masked by the fully formed glycerides. At first there is a marked accumulation of free fatty acids of low molecular weight with a comparatively low iodine number, which rapidly disappear as esterification begins, accompanied by a drop in the saponification value, indicating the presence of a much longer chain fatty acid.

The accumulation of the free fatty acids can be explained in one of two ways: (a) either the formation of glycerol begins after the formation of the acids, or (b) the enzymes necessary for the formation of esters are not present. In the early stages the degree of unsaturation is low and hydroxyl acids are present; later, concomitant with oil formation, the degree of saturation increases and the free acids largely disappear.

Summary

1. Beginning at approximately 90 days after flowering of Macadamia, embryo enlargement and accumulation of oil are very rapid; 70 per cent. of the oil is formed in 44 per cent. of the total growth period of the fruit.

2. The material utilized for oil formation moves into the fruit from outside sources. Oil synthesis and protein synthesis occur simultaneously.

3. In the early stages of oil synthesis short chain saturated fatty acids are formed first and accumulate; later, long chain unsaturated fatty acids are formed.

4. Hexose sugar is apparently the starting point for oil formation. From the sugar, short chain saturated fatty acids are formed. The short chain acids are converted into long chain unsaturated fatty acids as synthesis proceeds.

5. In the early stages of oil formation the short chain acids and glycerol do not combine probably because of the absence of the proper esterase.

HAWAII AGRICULTURAL EXPERIMENT STATION
UNIVERSITY OF HAWAII
HONOLULU, T. H.

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STIMULATIVE EFFECTS OF X-RAYS ON PLANTS

H. J. Kersten, H. L. Miller and G. F. Smith

(WITH ELEVEN FIGURES)

Introduction

The majority of previous investigations dealing with the effects of x-rays on plants have been concerned with the question of growth stimulation of the aerial portion of the plant resulting from treatments with weak doses of x-rays (**4, 5, 6, 8, 9, 13, 14**). The methods generally employed involved an x-ray treatment of unsoaked or soaked seeds, or of growing points. Observations recorded during subsequent growth, particularly at maturity, were examined for evidences of stimulation. Reports of the stimulative effects of x-rays on the growth of the aerial portions of the plants are numerous but in only a few cases is the evidence conclusive. Inadequate sampling and limited test periods have been given as reasons for regarding the evidence as inconclusive.

The view that a consistent growth stimulation effect might be obtained by using a specific x-ray dose, was emphasized by Shull and Mitchell (**21**). They employed metallic screens, high voltage, low amperage, and short exposures. The use of metallic screens eliminated the soft x-rays.

In addition to the x-ray treatment, however, it is also necessary to consider the condition of the test material. Chemical alterations, generally destruction, and re-synthesis are commonly affected by x-ray irradiation and, in biological materials, these may well be the fundamental factors concerned. Such x-ray-induced changes would undoubtedly alter plant growth in view of its chemical nature owing to the presence and activities of auxins, calines, growth inhibitors, and other growth factors (**18**). The experiments reported in this investigation were not designed to determine this chemical aspect of growth stimulation; it is worthy of consideration, however, in future experiments of this type.

It is the purpose of the work described here to investigate the question of stimulation of primary root growth in seedlings of seeds given a small dose of soft x-rays. Previous work of Rivera (**16, 17**) reported stimulative effects of treating roots with small doses of x-rays. This, however, was not a study on root growth stimulation directly, but rather on the resulting growth acceleration of aerial portions of plants following the irradiation of the roots.

In experiments described in this paper, corn was found to be most suitable for the method of germination employed. Stimulation as it will be used throughout refers to an increase in root length, or a greater wet or dry weight of roots of seedlings from treated seeds as compared with that occurring in the roots of normal seedlings. Observations were limited to the first five days of germination and the data presented refer solely to that period.

Methods

The x-ray radiation was provided by a gas type x-ray tube (**12**) having a copper target, operated at 10 ma. and at various voltages mentioned later. The seeds which were irradiated were arranged with embryos toward the source of radiation at a distance of eight cm. from the focal spot of the tube. The window of the tube, which was made of aluminum foil and Cellophane, transmitted the K_α and K_β characteristic radiation (1.54 and 1.38 Å.) as well as that of both longer and shorter wavelength radiation of the continuous spectrum of the copper target. The shorter wavelength emitted in

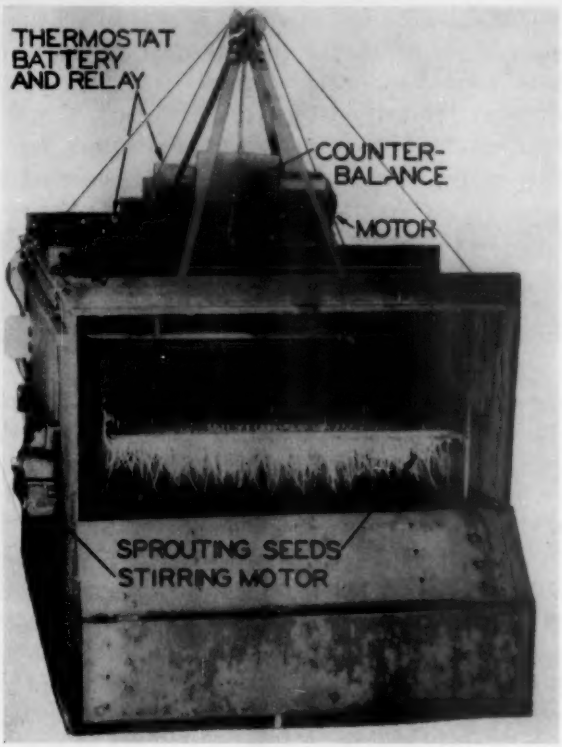

FIG. 1. Apparatus for seedling germination under controlled experimental conditions.

any experiment was approximately 0.5 Å. In every case, the time of exposure was 10 seconds.

After considerable preliminary work for the purpose of determining the requirements necessary to obtain sufficient data, it was decided to construct an apparatus which would permit 2000 seeds to be germinated simultaneously in water culture under controlled conditions. The apparatus (fig. 1) consisted of a large wooden cabinet in the bottom of which was a tank containing tap water kept at 29° C. by thermostatic control. A uniform

temperature of the water was maintained by the use of a stirring motor equipped with paddles to circulate the water in the tank. A constant flow of fresh water through the tank was made possible by small entrance and exit tubes.

The frame which supported the seeds during germination consisted of a rack suspended from above by four cables. The position of the rack below or above the water level was automatically controlled by means of a crank operated by an electric motor. Across the rack were placed 40 metal rods, each equipped with 50 fine nickel wire hooks. The purpose of each hook was to support a corn seed in its normal position by passing the hook through the endosperm portion of the seed at a point removed from the embryo.

The corn used in the experiments was Stowell's Evergreen Sweet Corn, 1940 crop. Badly formed and inferior kernels were discarded. The selected seeds were prepared for germination, the first part of which involved a twenty-four-hour soaking period, as follows: Fifty corn seeds were placed in each of forty copper wire baskets designed as seed-soaking containers. Twenty of these baskets of seeds were arbitrarily chosen as controls and the seeds in each of the remaining twenty baskets were given identical x-ray treatments.

It was found by trial that fifty roots, that is the content of one rod, could be examined for length and wet weight in a ten-minute interval. Consequently, in order to have all plants of the same age when observations on root growth were made, the following procedure was adopted: Fifty seeds were irradiated, replaced in a particular basket and the basket suspended from the rod to which the seeds were to be attached after soaking. Ten minutes later a basket containing non-irradiated seeds was suspended from the next rod. After another ten-minute interval a basket of irradiated seeds was suspended from the third rod. This procedure of starting the alternate soaking of control and irradiated seeds every ten minutes was continued for each of the 40 rods.

After a 24-hour soaking period the seeds were attached to the hooks of the rod which had suspended the basket. When the seeds were arranged on the wire hooks, a mechanism was started which automatically controlled the movement of the entire rack to positions below or above the water level. Preliminary experimentation indicated that good germination was possible if the germinating seeds were immersed in water for five-minute periods followed by two-hour intervals during which time they were suspended above the water.

At the end of the germinating period of four days, the rods were removed from the apparatus at ten-minute intervals in the same order as originally arranged, so that the age of the seedlings from the time that the seeds were first put to soak until they were harvested, was exactly five days. As each rod with its lot of seedlings was taken from the rack, the roots of the plants were severed as close to the seed as possible. The length of each root was measured and the total wet weight of the roots from the seedlings on this

rod was determined. The roots were then placed on paper and dried in the open air in the laboratory for seven days, after which time the total dry weight of the roots was determined. A seedling which did not produce root growth of at least two mm. in length was considered as not germinating.

Results

The results obtained show such a degree of variability that it is difficult to make a reliable interpretation of them without the use of statistical analysis. Tests of significance (7) were applied in comparing the experimental values obtained from the irradiated seeds with those obtained from the non-irradiated controls to determine whether the differences in these values could be ascribed, without much doubt, to a difference in the treatments received by the samples or whether they could be due to random sampling.

A comparison of the results obtained has been made with regard to the following variates:

The number of seeds germinating on each rod, x_1.
The total root length per rod, x_2.
The average root length per seed germinating on each rod, x_3.
The length of the longest root on each rod, x_4.
The total length of the 25 longest roots on each rod, x_5.
The total wet weight of roots per rod, x_6.
The average wet weight of root per seed germinating on each rod, x_7.
The total dry weight of roots per rod, x_8.
The average dry weight of root per seed germinating on each rod, x_9.

Each of the nine varieties was tested in two ways. First, the control seeds and irradiated seeds were regarded as separate and distinct samples chosen at random from the same population group. The difference between the mean value of a variate for the controls and for the irradiated seeds was tested to see if this difference was significant. Second, each rod of irradiated seeds was paired with an adjacent rod of control seeds. The differences of the variates for each pair was tested to see if its mean value differed significantly from zero. The first test led to a probability value p_1, which measured the probability of choosing from the same population group two random samples having mean values which differed by at least as much as that observed. The second test led to a probability value p_2 which measured the probability of choosing from a population group having a zero mean value a random sample having a mean value which differed from zero by as much or more than that observed. The usual convention of selecting a probability level of 5 per cent. or less as being significant has been adopted.

The percentage stimulation based upon the mean value of each variate for the control and for the irradiated seeds is given in Table I. The parentheses enclose those values for which one or both tests for significant dif-

TABLE I

PERCENTAGE STIMULATION OF VARIATE. BASED ON MEAN VALUE OF VARIATE

EXPERIMENT NUMBER	PEAK KILOVOLTS	r UNITS PER MINUTE	X_1 NUMBER OF SEEDS GERMINATING	X_2 TOTAL ROOT LENGTH PER ROD	X_3 AVERAGE ROOT LENGTH PER SEED GERMINATING ON EACH ROD	X_4 LENGTH OF LONGEST ROOT ON EACH ROD	X_5 TOTAL LENGTH OF 25 LONGEST ROOTS ON EACH ROD	X_6 TOTAL WET WEIGHT OF ROOTS PER ROD	X_7 AVERAGE WET WEIGHT OF ROOT PER SEED GERMINATING ON EACH ROD	X_8 TOTAL DRY WEIGHT OF ROOTS PER ROD	X_9 AVERAGE DRY WEIGHT OF ROOT PER SEED GERMINATING PER ROD
			%	%	%	%	%	%	%	%	%
1	20.0	1000	(6.2)*	(10.5)	(4.2)	3.0	(5.4)	(10.1)	3.7	(10.2)	4.0
2	15.0	450	− 1.1	2.6	3.9	2.8	2.3	2.5	3.2	2.6	3.3
3	0.0	0	−17.0	1.4	3.4	4.2	2.1	1.2	3.3	2.2	4.3
4	17.5	750	− 0.1	2.6	2.2	1.2	2.7	(8.6)	(8.3)	4.3	4.3
5	0.0	0	− 2.2	−1.3	0.1	−0.5	−1.8	3.2	(5.2)	0.6	2.6
6	22.5	1300	0.1	−0.8	−0.3	−1.1	−1.0	−3.1	−2.7	−0.6	−1.0
7	20.0	1000	(7.2)	(10.2)	2.2	1.4	(3.6)	(8.8)	1.2	(9.2)	1.4
8	25.0	1700	0.4	−1.6	−2.2	0.7	−1.0	−5.6	−5.7	−0.8	−1.6
9	20.0	1000	− 3.0	0.4	(3.3)	−1.5	1.0	0.6	3.7	−0.8	(3.3)

* Numbers in parentheses are those values for which one or both the tests for significance of differences indicated a real difference in the treatments of the controls and the irradiated seeds.

TABLE II

MOST PROBABLE LENGTH OF ROOT (15-MM. INTERVAL)

EXPERIMENT NUMBER	PEAK KILOVOLTS	r UNITS PER MINUTE	MIDPOINT OF INTERVAL		FREQUENCY		PERCENTAGE STIMULATION
			C	R	C	R	
							%
1	20.0	1000	101.0	112	172	183	10.9
2	15.0	450	94.0	102	154	148	8.2
3	0.0	0	86.0	89	104	221	3.5
4	17.5	750	75.0	86	185	202	14.7
5	0.0	0	71.0	74	171	180	4.2
6	22.5	1300	81.0	76	212	218	−6.2
7	20.0	1000	86.0	105	173	193	22.1
8	25.0	1700	130.0	127	159	133	−2.3
9	20.0	1000	119.5	127	216	201	6.3

ference indicated a real difference in the treatments of the controls and irradiated seeds. Except in one case, the only significant differences were obtained from samples irradiated at 17.5 to 20 peak kv. This one exception was in experiment 5 for which the voltage was zero. Since there was no difference in the treatments of those seeds marked "rayed" and those marked "control," this difference must be attributed to chance.

Using a 15-mm. class interval for the frequency tables of the root lengths of seedlings, the modal class for the seedlings from the control and irradiated seeds are given in table II. The percentage increases in the midpoint of this interval for the seedlings from the irradiated seeds over that for the control seedlings are given in this table. The averages of the percentage increases at zero kv. and 20 kv. are 3.8 and 13.1, respectively. The tests of significance do not indicate that the averages differ significantly from zero or from each other. The average of the increases at 17.5 and 20 peak kv. is 13.5 per cent. This value does differ significantly from zero but not from the results obtained at zero kv.

The percentages of stimulation at the various voltages are represented graphically in figures 2 to 11.

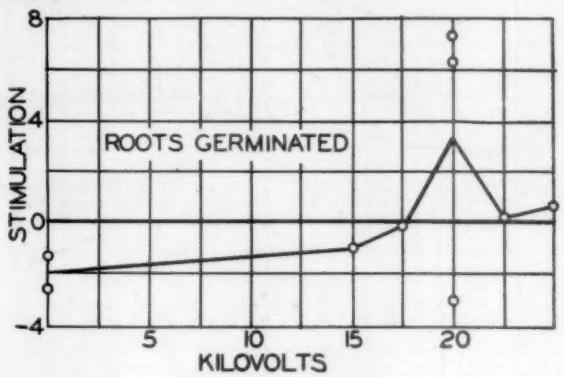

FIG. 2. Percentage stimulation determined from the number of seeds germinating on each rod (x_1) as a function of voltage.

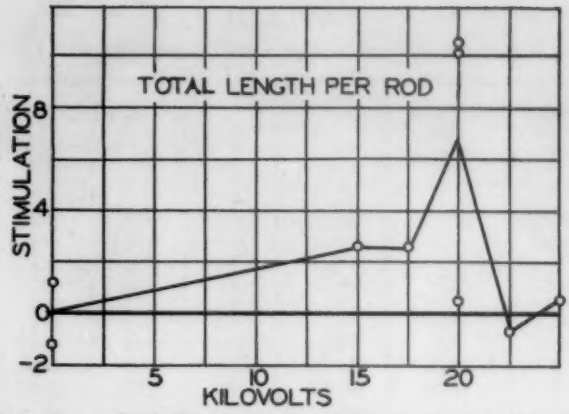

FIG. 3. Percentage stimulation determined from the total root length per rod (x_2) as a function of voltage.

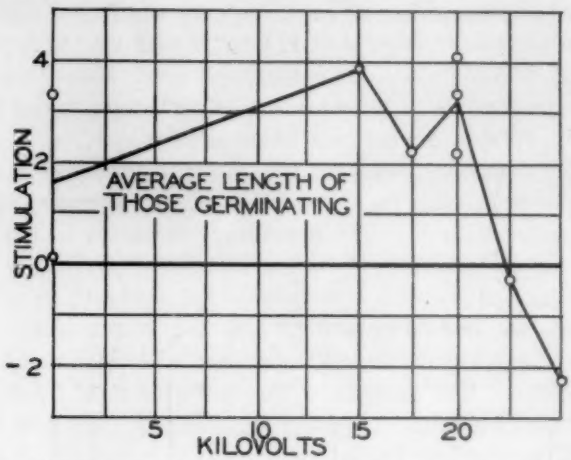

FIG. 4. Percentage stimulation determined from the average root length per seed germinating on each rod (x_3) as a function of voltage.

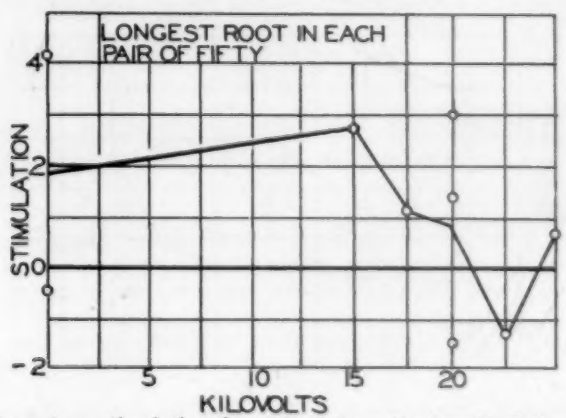

FIG. 5. Percentage stimulation determined from the length of the longest root on each rod (x_4) as a function of voltage.

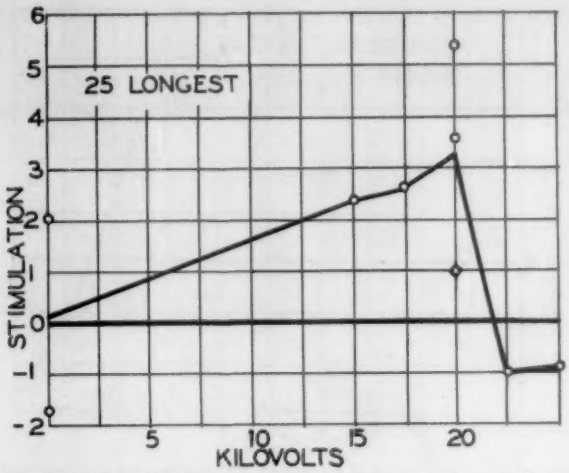

FIG. 6. Percentage stimulation determined from the total length on the 25 longest roots on each rod (x_5) as a function of voltage.

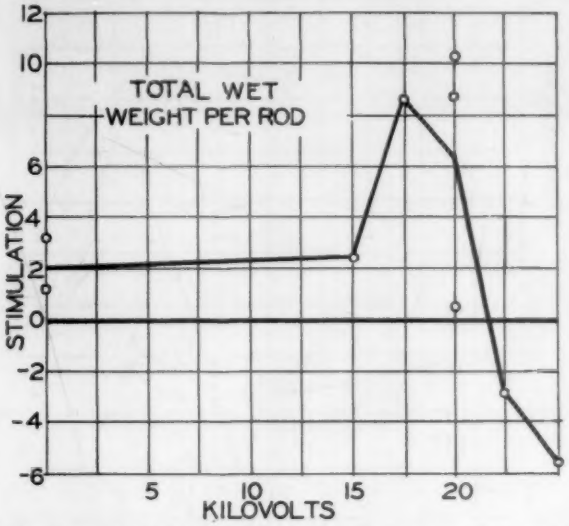

FIG. 7. Percentage stimulation determined from the total wet weight of roots per rod (x_6) as a function of voltage.

Discussion

No very great stimulation in primary root growth of corn seedlings from seeds given weak x-ray treatments as compared with that of control seedlings was obtained. Statistical analysis of the data, however, indicates beyond a reasonable doubt that the treatment of these seeds by x-rays employing a voltage in the neighborhood of from 17.5 to 20 peak kv. did produce a significant increase in the average length and in the wet and dry weights of the roots of the seedlings. Voltages of 22.5 and 25 peak kv. did not produce a significant change in any of the varieties examined.

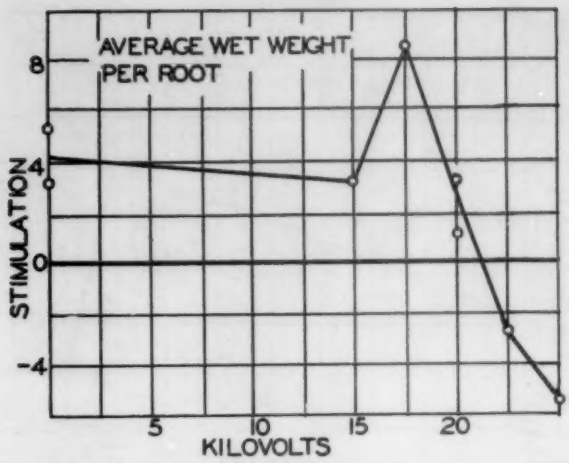

FIG. 8. Percentage stimulation determined from the average wet weight of root per seed germinating on each rod (x_7) as a function of voltage.

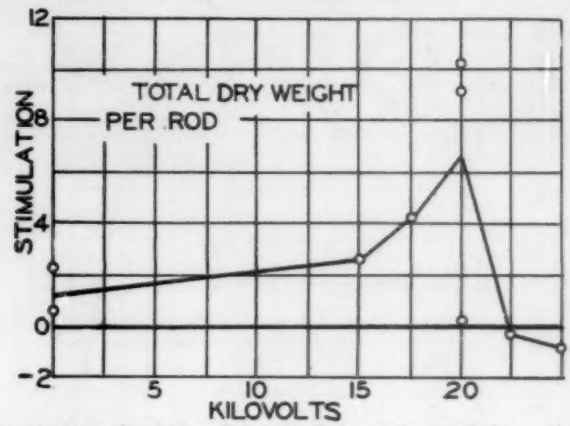

FIG. 9. Percentage stimulation determined from the total dry weight of roots per rod (x_8) as a function of voltage.

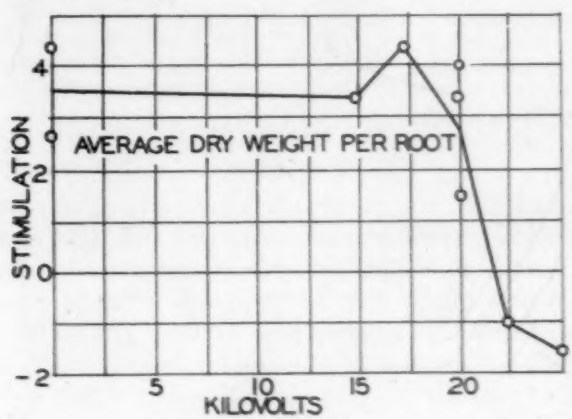

FIG. 10. Percentage stimulation determined from the average dry weight of root per seed germinating on each rod (x_9) as a function of voltage.

It is suggested by the data that the amount of radiation as well as the condition of the biological material to be treated which might induce growth stimulation may be very sharply defined. A slight change from the optimum condition for growth stimulation may fail to produce sufficient stimulation to detect or may even induce growth inhibition.

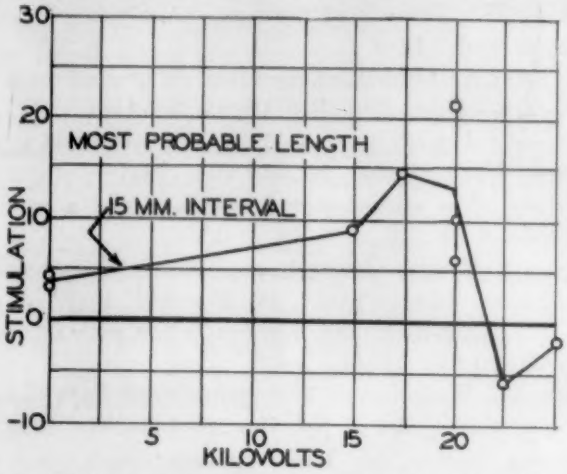

FIG. 11. Most probable root length (15 mm. intervals) as a function of voltage.

Summary

1. An apparatus for seedling germination which fulfills requirements of adequate sampling necessary to obtain reliable conclusions in investigations of x-ray induced growth stimulation has been described.

2. Under the conditions of these experiments it is possible to obtain an apparent x-ray-induced stimulation in the primary root growth of *Zea mays* seedlings. Statistical analysis of the data obtained indicates beyond a reasonable doubt that stimulation of root growth was obtained by irradiating dry seeds at voltages in the neighborhood of 17.5 to 20 peak kv. The analysis shows that differences as much as those observed would occur in fewer than five cases in one hundred trials if the x-ray treatment made no essential difference in root growth.

UNIVERSITY OF CINCINNATI
CINCINNATI, OHIO

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EFFECTS OF DROUGHT, TEMPERATURE AND NITROGEN ON TURF GRASSES[1]

J. C. CARROLL

(WITH ONE FIGURE)

Introduction

The factors of major importance in the distribution of grasses are moisture and temperature, and observations have shown that management plays an important part in the duration of grasses. The present investigation was initiated to determine: (a) the relative survival of a number of turf grasses under different degrees, or intensities, of moisture and temperature, and (b) the effect of nitrogenous fertilization on these responses.

NEWTON and MARTIN (**21**) in a study of drought resistance of some crop plants in southern Alberta, including a number of grasses, found that bound water content could be used as a basis of classifying many of these plants in order of their drought resistance. On the other hand WHITMAN (**30**), working with four representative grassland types in western North Dakota, was unable to correlate bound water content with the ability of the species to withstand drought. VASSILIEV (**27**), VASSILIEV and VASSILIEV (**28**) believed that sugars play an important rôle in the drought resistance of wheat. TUMANOV (**26**) showed that repeated wilting of sunflowers resulted in increased hardiness to drought, and similar results with wheat plants were obtained by AAMODT and JOHNSTON (**2**).

BRIGGS and SHANTZ (**5**) in their review of previous work, state that many investigations have shown the water requirement of plants to be reduced by the use of fertilizers, the reduction being marked on poor soils, but slight on fertile soils. In a search for methods to eliminate quack grass, DEXTER (**10**) found that heavy fertilization with nitrogen lowered its resistance to drought. DEXTER (**9**) and CARROLL and WELTON (**7**) found that high-nitrogen plants failed to harden to cold as much as low-nitrogen plants.

MAXIMOV (**15**) in his investigations came to the conclusion that the true measure of drought resistance is the ability of the plant to survive exposure to drought without permanent injury. NEWTON (**19, 20**) found that he could distinguish cold-hardy from non-cold-hardy varieties of wheat on the basis of their bound water content. He also observed the higher sugar content of the hardy varieties as compared with that of the non-hardy, but assigned no particular significance to it. On the other hand, CHANDLER (**8**), and later ÅKERMAN (**3**), MEYER (**17**), and MAXIMOV (**15**), stressed the idea that soluble carbohydrates may increase frost resistance of plants by decreasing the precipitation of proteins.

Among recent investigators (**15, 18**) it appears to be the consensus of

[1] Published with the approval of the Director of the Ohio Agricultural Experiment Station.

opinion that drought and cold resistance cannot be accurately predicted by any of the physico-chemical methods yet devised, and that for the most reliable results the method of direct testing should be employed. Equipment used in making drought studies has been described by SHIRLEY (**24**), AAMODT (**1**), and BAYLES, TAYLOR, and BARTEL (**4**), and use of artificial refrigeration in cold studies has won general acceptance.

Materials

The grasses included in the present investigations were: Kentucky bluegrass (*Poa pratensis* L.), Canada bluegrass (*P. compressa* L.), roughstalked meadow grass (*P. trivialis* L.), wood meadow grass (*P. nemoralis* L.), annual bluegrass (*P. annua* L.), Astoria bent and Cocoos bent (*Agrostis tenuis* L.), South German mixed bent (*A. tenuis* L., *A. palustris* L., and *A. canina* L.), Highland velvet bent (*A. canina* L.), red fescue (*Festuca rubra* L.), Chewings fescue (*F. rubra fallax* L.), sweet vernal (*Anthoxanthum odoratum* L.), perennial rye (*Lolium perenne* L.), Italian rye (*L. multiflorum* L.), crested dogstail (*Cynosurus cristatus* L.), redtop (*Agrostis alba* L.), and large crabgrass (*Syntherisma sanguinale* (L.) Dulac). Although desirable, the seed of the species used was not genetically pure; and it is recognized that tests similar to those reported in this paper should be made of the many strains within each species. The plots 4 by 50 feet were first seeded April 26, 1938, and reseeded September 6, 1939. The soil was a Wooster silt loam and in a high state of productivity. The results of the soil drought and high temperature tests reported here were obtained from the later seeding, while those of the low temperature and physico-chemical tests were made on the grass of both seedings. Owing to weather conditions at Wooster during the summer and early fall of 1940 neither soil nor air temperatures were extreme. Thus the turf samples subjected to soil drought and high temperatures were not hardened naturally at the time of sampling. The samples exposed to soil drought may have hardened while drying.

The plats were maintained under lawn conditions and were divided into two equal sections as follows: (1) Low nitrogen— no treatment: and (2) High nitrogen—ammonium sulphate applied at rate of 5 lb. per 1000 sq. ft. in April, July, and September.

GENERAL METHODS

A golf-green cup-hole cutter was used to cut samples of sod from the plats. By means of this tool "plugs" of sod 4¼ inches in diameter and approximately 3 inches in thickness were lifted from each plat and placed in glazed earthenware jars of the same dimensions. Before the samples were transferred to the jars, the plugs were "sized" with a knife so that the surface of the soil was approximately one-fourth inch below the edge of the jar. As a result of this procedure an approximate uniformity of soil mass among the samples was obtained, and they could be watered easily at any time. The grasses were clipped either with a lawn mower just previous

to sampling, or with shears afterward. The plugs of sod were subsequently exposed to different degrees of drought and temperature under experimental conditions. The dry weight of each plug was estimated by sampling the adjacent soil for moisture determinations. These estimates proved to be reliable when tested by the dry weights of the plugs at the conclusion of several of the treatments. Occasional tests of moisture in the plants were also made during the period of the investigation.

In the preliminary tests, the percentage of survival was obtained by counting the number of living and dead plants at the end of 3 weeks after each test was completed. Each stem was considered as a separate plant. It was perceived that such a method of evaluating results would be too time-consuming when a full complement of samples was taken. Therefore, in order to shorten the time required to evaluate results, the percentage of survival on a limited number of samples was estimated first by the writer and then one other person. These estimations were followed by actual counts. The difference between the percentages of survival obtained by count and by estimation was not significant, and the latter method was used in the evaluation of results reported in this paper.

Soil drought

The relative survival of the different grasses when exposed to soil drought was tested by permitting the soil to dry slowly and exposing the samples to an air temperature of 35° C. for 4 hours daily until the moisture of the soil had reached 5 per cent. in one series and 3 per cent. in a second series, on a dry-weight basis. These values are below the wilting coefficient of Wooster silt loam, which has been found to be about 7.7 per cent. The time of drying varied from 7 to 8 days for the 5 per cent. series and was approximately 2 days longer for the 3 per cent. series. The moisture content of the soil was ascertained by weighing daily until the end point was nearly reached, then weighing more frequently. To insure uniform drying below the wilting point the plugs were raised and supported to allow circulation of air on all sides.

These conditions of soil moisture and air temperatures were chosen because they are similar to those occasionally encountered in the field in this section of the United States. During periods of drought at Wooster in the summers of 1931 and 1936, the moisture in the first 4 inches of soil beneath Kentucky bluegrass sod became as low as 3.2 per cent.

In late summer, drying took place in the greenhouse, where the air temperature was maintained at approximately 35° C. for 4 hours. In the fall, when the greenhouse temperatures were generally below 25° C., the samples were placed in a Freas draft oven at 35° C. for 4 hours late in the day. By this procedure the later samples were not exposed to a materially shorter day than were the samples tested earlier in the season. There was no essential difference between results obtained by greenhouse and oven heating of samples. LAUDE (**12**) has shown that in plants previously exposed to light, the heat resistance is lost very gradually.

After the percentage of water in the soil of the two series has been reduced to 5 per cent. and 3 per cent., respectively, sufficient water was added to bring the moisture content to approximately 70 per cent. of the water-holding capacity of the soil, and at the end of 3 weeks, an estimation was made of the percentage of recovery. This percentage of recovery is considered an index of the relative drought resistance of the different species of grasses studied (table I).

The results of the tests on soil drought at the two percentages of moisture content show that at 5 per cent. there was little or no injury among the grasses from the low-nitrogen section with the exception of *Cynosurus cristatus* (table I). The majority of the species from the high-nitrogen

TABLE I

SURVIVAL OF CERTAIN TURF GRASSES AS AFFECTED BY THE NITROGEN AND WATER CONTENT OF THE SOIL

GRASS	PERCENTAGE OF SURVIVAL*			
	5 PER CENT. SOIL MOISTURE		3 PER CENT. SOIL MOISTURE	
	LOW-N SECTION	HIGH-N SECTION	LOW-N SECTION	HIGH-N SECTION
	%	%	%	%
Poa pratensis	100	70	80	50
Festuca rubra fallax	100	90	75	35
Agrostis tenuis (Cocoos)	100	40	75	40
A. tenuis (Astoria)	95	70	70	45
A. canina (Highland)	100	50	70	40
Festuca rubra	90	80	65	45
So. German mixed bent	80	80	60	40
Syntherisma sanguinale	80	.....	60	.....
Agrostis alba	90	90	55	50
Poa compressa	80	55	50	35
P. annua	85	70	50	35
Lolium perenne	.....	.....	50	.....
Anthoxanthum odoratum	90	80	45	30
Poa trivialis	80	80	40	35
P. nemoralis	80	60	35	15
Cynosurus cristatus	60	30	20	20

* Average of estimations in 6 tests.

section, however, were definitely injured. At 3 per cent. moisture, marked differences among the species from both the low- and high-nitrogen sections were obtained (table I). Since approximately 2 days more were needed to reduce the soil moisture to 3 per cent. than to 5 per cent., it is highly probable that the time factor may be of more significance than the decrease of soil moisture from 5 to 3 per cent., for both these percentages are below the wilting coefficient of Wooster silt loam.

The effect of soil drought was tested six different times, and the results were, in general, very consistent. Among all the species tested, *P. pratensis* was consistently the most resistant, and *C. cristatus* was the least resistant to soil drought. As might be expected, there was considerable variation

among the species of each genus. In the Poa genus, *P. pratensis* far outranked others of the group included in this test on soil drought. On the basis of past observations on our own turf plats, as well as from reports in the literature, *P. trivialis, P. nemoralis,* and *P. annua* were not expected to withstand a high degree of soil drought, and this expectation was amply fulfilled in the present tests. *P. compressa* has been reported as being more drought resistant than *P. pratensis* (**22**) but in the tests made during this study, it was a very poor second, ranking only slightly above *P. trivialis,* which is notoriously non-hardy under drought conditions.

Different reasons have been assigned in the literature as to why either *P. pratensis* or *P. compressa* dominates any given locality to the virtual exclusion of the other, but there seems to be little agreement as to causes. BROWN (**6**) in testing four pasture grasses, including *P. pratensis* and *P. compressa,* under controlled temperature conditions, found that when both air and soil temperatures were maintained at 100° F. for approximately 2 months, *P. compressa* was injured much more than *P. pratensis.* He concluded that the destructive effects resulted more from a high soil temperature than from a high air temperature.

During the periods in which the writer's samples were exposed to an air temperature of 35° C., the soil temperature eventually equaled the air temperature. The highest soil temperature attained in this test was lower than that in the test by BROWN (**6**), and maintained for a considerably shorter period, but the relative amount of injury in the two grasses was similar. In the present experiments, therefore, it appeared that the effect was due chiefly to soil drought, and not to high soil temperature.

The species of the genera Agrostis and Festuca which were tested did not differ materially among themselves, and, as can be seen from table I, there was little difference between these two groups and *P. pratensis,* with the exception of South German mixed bent and *A. alba,* which were injured much more than the other grasses in the bent group.

The relatively high survival of the fescues was expected, for experience and observation had shown them to be fairly resistant to drought. On the lawn plats at Wooster, one plat of Chewing's fescue, located on the south side of a maple tree, has maintained a good stand since being seeded in 1928, with no attention other than being mowed at regular intervals. In that span of years, there have occurred several periods of extreme drought, but the stand of Chewing's fescue has not been seriously affected. This species is a valuable ground cover in locations where low moisture conditions exist, as on terraces. In contrast, *P. trivialis,* located on an adjacent plat, has had to be reseeded annually, since it has died out each season within the space of a few weeks after the inception of hot, dry weather. In a study of the water-supplying power of soil under different grasses, WELTON and WILSON (**29**) found that fescues apparently absorb less of the available soil moisture than do bluegrass and bents, and WILSON and LIVINGSTON (**31**) in a study of the wilting of grasses as related to the water-supplying power of

the soil found the fescues to be more drought-resistant than the other species tested.

Of the other grasses included in this study, *A. alba* and *L. perenne* are the most important from the standpoint of their value in pastures and in production of turf. The fact that they rank low in survival when exposed to soil drought may partially account for their gradual elimination by other grasses generally included in turf mixtures.

The effect of liberal applications of nitrogen on the survival of the different grasses on dry soil is shown in table I. In all cases, the recovery of the grasses from the high-nitrogen section was less than that from the low-nitrogen section, and in most cases the difference was marked (fig. 1). In a few cases only, was there a significant difference in the percentage of survival among the grasses from the high-nitrogen section.

Effect of hardening on resistance to soil drought

To determine the effect of repeated wilting on the drought hardiness of grasses, duplicate samples of five species representing four genera were taken on one of the dates when all the species were sampled for the usual tests. The same procedure was followed as in the regular tests, with the exception that when the soil was dried to approximately 5 per cent. moisture, sufficient water was added to bring it back to the original moisture condition, after which it was left to dry to 3 per cent.

The results (table II) show that with *P. pratensis* and *F. rubra fallax*, there was no increase in drought hardiness, but that in *P. compressa* and *C. cristatus*, an increase in hardiness was obtained by one previous drying. *P. pratensis* and *F. rubra fallax* probably harden more quickly than the other grasses tested.

High soil and air temperatures

It has been found by numerous workers that large fluctuations in soil temperature are confined chiefly to the upper layers, also that on bare soil in summer, the temperature of the upper soil layer is generally greater than

Fig. 1. Effect of soil drought on low- and high-nitrogen grasses at 3 per cent. soil moisture content.

Jar no.	Grass	Treatment
1	*P. pratensis*	Low N
2		High N
3	*P. compressa*	Low N
36		High N
9	*P. annua*	Low N
10		High N
11	*A. tenuis* (Astoria)	Low N
12		High N
17	*A. canina* (Highland)	Low N
60		High N
23	*Anthoxanthum odoratum*	Low N
24		High N
27	*A. alba*	Low N
28		High N

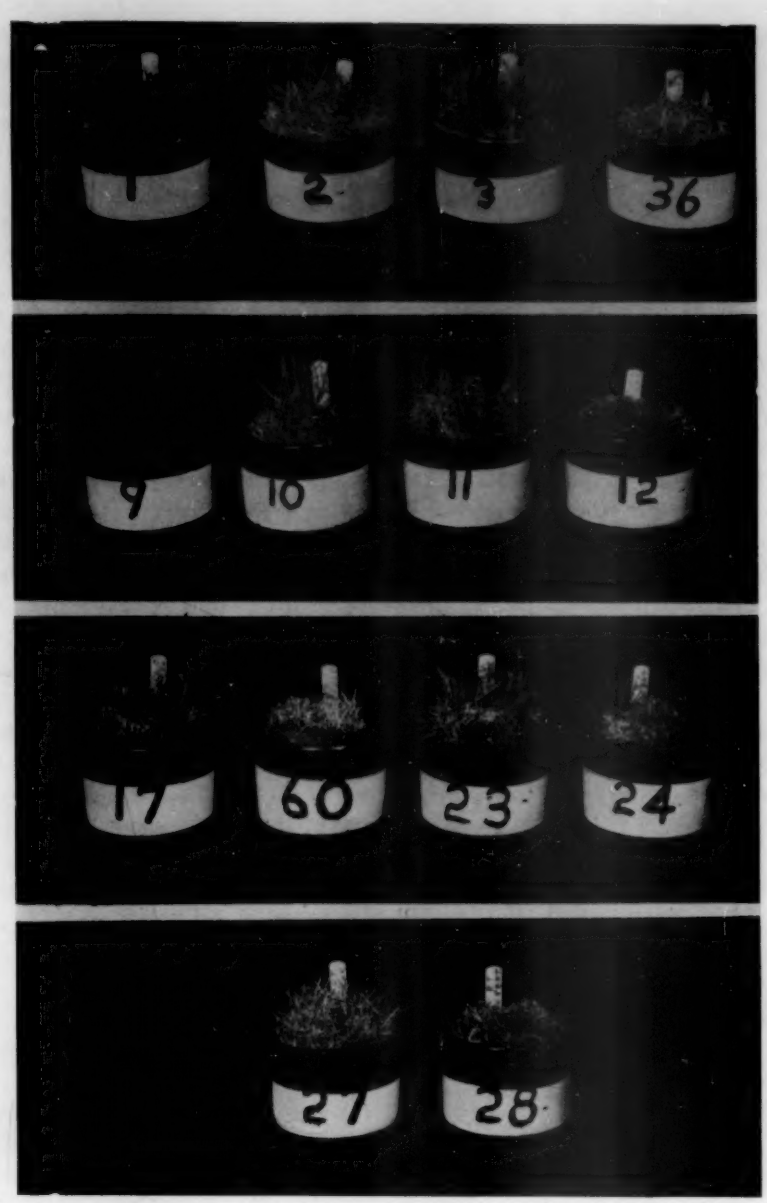
1
2
3
36
9
10
11
12
17
60
23
24
27
28

TABLE II

COMPARATIVE EFFECTS OF REPEATED DRYING VERSUS SINGLE DRYING ON SOIL DROUGHT ENDURANCE OF CERTAIN TURF GRASSES

GRASS	PERCENTAGE OF SURVIVAL AFTER DRYING TO 3 PER CENT.	
	SINGLE DRYING	REPEATED DRYING*
	%	%
P. pratensis	80	80
P. compressa	50	80
A. tenuis (Astoria)	70	50
F. rubra fallax	75	75
C. cristatus	20	60

* Soil dried to approximately 5 per cent. moisture, watered, and then dried to 3 per cent.

that of the air. SMITH (**25**) reported temperatures of 146° F. and 107° F., respectively, at ½-inch and 3-inch depths in bare soil with an air temperature of 105° F. Reports in the literature on soil temperature beneath sod are very fragmentary. FITTON and BROOKS (**11**), in a survey of soil temperature studies, list only a few readings taken beneath sod. The maximum temperature during August under a bluegrass sod at a 2-inch depth was 73.2° F.; the air temperature was not given. In an orchard sod at a depth of 3 inches, the temperature was 76.4° F. with an air temperature of 70.2° F.; the kind of grass was not named.

Records of air temperature at different heights above the soil surface are more numerous and more complete than those of soil temperature. These include measurements taken above different types of cover. Temperatures of 105° F. to 115° F. have frequently been reported in the central section of the United States. ROBB (**23**) at the Topeka, Kansas, Weather Bureau has reported air temperatures as high as 116° F. at a height of 5 feet above sod.

Owing to weather conditions at Wooster during the summer season of 1940, neither soil nor air temperatures were extreme. The summer season was above average in rainfall and below average in temperature, with only about 2 weeks of hot, dry weather which occurred during the latter half of July. Soil temperatures at a depth of 2 inches on the turf plats rose during that period to a maximum of 86° F. at 2:00 P.M., July 22, with an air temperature of 91° F.

The temperature of the soil beneath the grasses on the high-nitrogen section was invariably lower than that on the low-nitrogen section, due, no doubt, to the higher moisture content of the soil in the high-nitrogen section.

HIGH SOIL TEMPERATURE

To determine the effect on turf grasses of high soil temperature accompanied by high soil moisture, 60 jars of sod representing 4 replications of each grass were placed on a tray in a steel tank containing water heated by

means of a steam coil. The soil temperature was measured by a thermometer inserted to a depth of 1½ inches in the center of each plug of sod. The water was circulated while being heated in order to maintain an even temperature throughout the tank. Within about 2 hours, the temperature of the soil became the same as that of the surrounding water. In one test, the soil temperature was held at 50° C. for 4 hours; in the other test, the jars were removed as soon as the maximum temperature was attained. The results of subjecting the grasses to a high soil temperature are recorded in table III.

Soil temperatures frequently influence plant survival more than air temperatures. Since the high soil temperature in these experiments was not accompanied by a high air temperature, the initial injury in this case was limited to the underground portions of the plant. As the moisture content of the soil was comparatively high, the injury could not have been due to desiccation. The experiment was not continued long enough to bring about a condition of starvation through increased respiration. It appears, therefore, that the injury was due to thermal effects upon the protoplasm. From data available in the literature, it appears that most non-dormant plant cells are heat-killed by temperatures of 50° C. to 60° C., sometimes even lower. The lethal temperature is lower with a slow rise in temperature than with a comparatively more rapid rise (**13**).

Merely bringing the soil temperature to 50° C. was not lethal to the grasses growing in the low-nitrogen section, but most of these same species in the high-nitrogen section were injured by this treatment (table III). Where the grasses were held at 50° C. for 4 hours, the injury was much greater

TABLE III

SURVIVAL OF CERTAIN TURF GRASSES AS AFFECTED BY THE NITROGEN CONTENT AND HIGH TEMPERATURES OF THE SOIL

GRASS	PERCENTAGE OF SURVIVAL					
	50° C.*		60° C.*		4 HR. AT 50° C.	
	LOW-N SECTION	HIGH-N SECTION	LOW-N SECTION	HIGH-N SECTION	LOW-N SECTION	HIGH-N SECTION
	%	%	%	%	%	%
Syntherisma sanguinale	.....	.....	.....	.....	85	.....
A. canina (Highland)	100	100	20	2	70	15
F. rubra fallax	100	90	70	0	65	10
P. trivialis	100	90	5	5	60	20
P. annua	100	70	20	0	60	55
F. rubra	100	90	25	0	45	7
P. compressa	100	100	70	50	40	20
A. tenuis (Cocoos)	100	70	1	0	40	7
A. alba	100	80	0	0	40	5
P. pratensis	100	100	2	2	25	40
Anthoxanthum odoratum	100	60	10	0	20	1
P. nemoralis	100	65	2	0	15	1
So. German bent	100	70	1	1	15	5
Cynosurus cristatus	100	100	0	0	15	5
A. tenuis (Astoria)	100	50	0	2	3	2

* Jars removed as soon as maximum soil temperature was attained.

(table III). Thus, the lethal temperature varies with the time of exposure. In all three experiments, the time necessary to bring the soil temperature to the maximum was approximately 2 hours. From the data in table III, it is evident that the underground portions of a number of these grasses cannot long endure soil temperatures between 50° and 60° C. The greater injury occurred in the grasses from the high-nitrogen section.

Results indicate that *P. compressa* is better able to withstand high soil temperatures than *P. pratensis*. This result is in contrast to the results obtained by BROWN (**6**), who in a study of the effect of combined high soil and air temperatures on the growth of the two grasses, found that *P. compressa* was injured more than *P. pratensis*. He concluded that the effect was due to high soil temperature rather than to high air temperature. In his tests, however, the grasses were kept at a soil and air temperature of 100° F. for approximately 8 weeks, and a starvation effect due to a high rate of respiration was probably obtained. In the test reported here, the temperature of 50° C. (122° F.) for 4 hours probably produced a more direct killing effect. Owing to its habit of growth, *P. compressa* shades the soil less than *P. pratensis*, and one would expect higher daily soil temperatures on some of the sites where *P. compressa* dominates. Its relatively more frequent dominance on shallow soils above rocks adjacent to deeper soil areas dominated by *P. pratensis* is probably related to its endurance of short periods of high soil temperatures and low fertility rather than of drought.

A. canina (variety Highland), which was found to be fairly resistant to soil drought, was the most resistant to high soil temperature (50° C. for 4 hours) of any of the grasses tested, and *F. rubra fallax* ranked second. The other bent grasses were much less resistant to high soil temperatures than was Highland; but tests later in the fall showed that they survived low temperatures much better than Highland. It appears, therefore, that the changes in the inner conditions of plants which enable them to endure extremes in temperature are not necessarily the same for both heat and cold. This difference is further borne out in a limited way by the results of measurements of bound water which show no significant difference between grasses representing extremes in drought, heat, and cold endurance (table VII). These data further substantiate the argument of those who have declared the inadequacy, at the present time, of physico-chemical analyses as reliable indices of the relative resistance of plants to extreme conditions of temperature and moisture.

HIGH AIR TEMPERATURES

The effect of short exposures to high air temperatures on turf grasses was investigated by placing the grasses for different intervals of time in a Freas draft oven. The temperatures used were 40°, 50°, and 60° C., and the time, 4 and 6 hours at each temperature. The exposure to heat was made in the evening, as in the experiments on soil drought. In this test, however,

the moisture content of the soil remained relatively high, but the relative humidity of the air passing over the samples was appreciably lower (approximately 15 per cent. at 50° C.) than obtained in the test on soil drought. Four replicates of each grass were tested.

In this experiment, as in the test on high soil temperatures, the results were doubtless due to thermal effects. However, in exposing the grasses to air temperatures similar to those used in the high soil temperature tests, much less injury was obtained. The reason for this cannot be stated definitely. With some grasses, the extent and type of root development may be the explanation; with others, the presence of rhizomes enabling the plant to send up new shoots when the original aerial portions have been destroyed may be of greater importance. Again, as has been suggested by MAXIMOV

TABLE IV

SURVIVAL OF CERTAIN TURF GRASSES WHEN EXPOSED TO AN AIR TEMPERATURE OF 50° C. FOR 6 HOURS

GRASS	PERCENTAGE OF SURVIVAL	
	LOW-N SECTION	HIGH-N SECTION
	%	%
P. pratensis	80	40
A. tenuis (Cocoos)	70	20
A. canina (Highland)	70	55
A. tenuis (Astoria)	65	15
P. trivialis	60	35
P. annua	60	40
Agrostis alba	60	20
P. compressa	50	2
So. German mixed bent	50	25
Syntherisma sanguinale	45	....
Anthoxanthum odoratum	40	2
F. rubra fallax	20	5
F. rubra	20	7
P. nemoralis	10	5
Cynosurus cristatus	7	2
Final soil temperature	40.1° C.	

(16), the ability of the stomatal mechanism to withstand high temperatures may play an important part in the heat resistance of a plant.

The effects on the grasses of heating at 40° C. for 4 and 6 hours were negligible; there was only a slight injury to the tips of some leaves on both low- and high-nitrogen sections. In the test at 50° C. for 4 hours, there was very little injury to any of the grasses on the low-nitrogen section, but there was much greater injury to the grasses on the high-nitrogen section. Of all the exposures, the greatest difference among the grasses was obtained at 50° C. for 6 hours (table IV). The species of Poa and Agrostis used in this investigation, with the exception of *P. nemoralis* and South German mixed bent, withstood high air temperatures better than the species of the other genera tested. With few exceptions, those grasses having the greater resistance to soil drought also endured high air temperature better than

those with a lower resistance to soil drought. The exceptions particularly noted were the fescues, which were much less resistant to high air temperature than to soil drought. These results are at variance with those observed under outdoor conditions, but it is extremely unlikely that the low relative humidity prevailing in this test and the limited amount of soil would be duplicated under natural conditions.

Low temperature

The extent of injury to a plant due to cold depends upon the type of plant and upon the nature of its preconditioning. Most perennial plants

TABLE V

Percentage of survival of turf grasses following a single cooling to different soil temperatures

Grass	Percentage of survival with a final soil temperature of							
	−5° C.		−10° C.		−15° C.		−20° C.	
	Low-N	High-N	Low-N	High-N	Low-N	High-N	Low-N	High-N
P. pratensis	100	90	80	60	25	5	20	5
P. compressa	90	80	30	30	5	2	.....	.....
P. trivialis	90	50	45	25	0	0	0	0
P. nemoralis	80	30	70	5	0	1	.....	.....
P. annua	80	50	40	10	3	1	5	0
A. tenuis (Astoria)	90	80	60	50	0	0	0	0
A. tenuis (Cocoos)	90	75	80	45	1	0	0	0
So. German mixed bent	90	75	70	50	0	0	0	0
A. canina (Highland)	100	20	5	5	0	0	.....	.....
A. alba	90	90	60	40	0	0	0	0
F. rubra fallax	90	65	75	30	0	0	0	0
F. rubra	80	65	60	25	0	0	0	0
Anthoxanthum odoratum	50	30	20	10	0	0	0	0
Cynosurus cristatus	40	20	0	0	0	0	0	0
L. perenne	70	20	0	0	0	0	0	0
L. multiflorum	40	10	10	0	0	0	0	0

growing in the temperate region can, to some extent, be hardened to cold and are thus enabled to withstand lower temperatures than when not hardened. In the present study, a comparison of the endurance of different grasses to cold was made on samples from both the unfertilized and the fertilized sections of the plats. The grasses were permitted to harden naturally. Previous work (**7**) had shown that air temperatures near the freezing point were necessary to harden turf grasses. That nitrogen treatments may affect the degree of hardening of turf grasses to cold was shown by Carroll and Welton (**7**).

The technique employed to test the survival of species of turf grasses

exposed to low temperature by artificial refrigeration was essentially the same as that described previously (**7**). Samples from the low- and high-nitrogen sections of each grass were placed in the freezing chamber at −25° C., and as soil temperatures of −5°, −10°, −15°, and −20° C., were reached, duplicate samples of each grass for each treatment were removed. The samples were then placed in the greenhouse and after 3 weeks, an estimation was made of the percentage of survival.

The results obtained by exposing the grasses to cold until definite soil temperatures were attained are shown in table V and represent 6 replications. Only three grasses from the low-nitrogen section were appreciably injured at a soil temperature of −5° C., but injury of the high-nitrogen grasses was more general. This difference between grasses from the low- and high-nitrogen sections held throughout the temperature range of the test, except where the injury of a particular species was complete in both sections. *P. pratensis* was found to be the most resistant to low temperatures of any of the grasses tested. However, within the range of low soil temperatures generally encountered in the field at Wooster, *F. rubra fallax* and some of the Agrostis species survived nearly as well as *P. pratensis*.

Soil temperatures beneath sod at Wooster during the winter of 1940–1941 were not low enough to injure any of these turf grasses except the least cold-resistant ones on the high-nitrogen sections. At a depth of 1½ inches, the soil temperature was not lower than −2° C., as the ground was covered by a thin covering of snow during the coldest periods. MAIL (**14**), in studying the effect of soil temperatures on insect survival, found that under a light covering of snow (¾ inch to 2 inches), the soil temperature remained within 1° or 2° of the freezing point, whereas on sod-covered ground, without snow cover, a soil temperature of −10° C. was recorded at a depth of 2 inches, when the air temperature was −13° C.

Since the degree of hardening of turf grasses to cold was decreased on the sections to which high amounts of nitrogen had been applied, it would appear inadvisable to supply grass with any considerable amount of this fertilizer constituent late in the fall. The time of application should, of course, be determined by the prevailing weather conditions, so that a luxuriant growth late in the season would be avoided. BROWN (**6**) found that Kentucky and Canada bluegrass and orchard grass made considerable growth at 40° F. In a study of the comparative effect of different carriers of nitrogen on growth of Kentucky bluegrass, WELTON and CARROLL (unpublished data) found little diminution of the weekly absorption of nitrates in late fall when the daily maximum and minimum air temperatures were, respectively, slightly above and below 40° F.

EFFECT OF SOIL DRYING ON HARDENING TO COLD

Statements have appeared in the literature (**26**) to the effect that decreasing the moisture supply of plants facilitates their hardening to cold. To test whether this statement might be true of turf grasses, two lots of five

species were hardened to cold; one lot with an optimum soil moisture, and the other with a soil moisture slightly above the wilting percentage. After hardening by daily exposure to a temperature of approximately 0° C. for 1 week, the grasses were placed in a freezing chamber and removed as soon as the soil temperature had been lowered to – 10° C. The time necessary to cool the soil to this minimum temperature varied from approximately 3½ hours for the samples with high moisture content to 1½ hours for those with the low moisture content. The results (table VI) show that drying of the

TABLE VI

COMPARATIVE EFFECTS OF HARDENING BY COLD ONLY VERSUS HARDENING BY COLD PLUS SOIL DRYING ON THE COLD-ENDURANCE OF CERTAIN TURF GRASSES

GRASS	PERCENTAGE OF SURVIVAL AFTER FREEZING TO – 10° C.*		
	UNHARDENED	HARDENED BY COLD	HARDENED BY COLD PLUS SOIL DRYING (SINGLE DRYING)
	%	%	%
P. pratensis	0	80	75
P. compressa	0	30	45
A. tenuis (Astoria)	0	60	15
F. rubra fallax	0	75	25
C. cristatus	0	0	10

* – 10° C. soil temperature.

soil resulted in no significant gain in cold hardiness by three of the grasses and was detrimental to the remaining two. On the latter, the greater cold injury may have been due to the more rapid lowering of the soil temperature, and not to the extremity of the minimum temperature itself.

Physico-chemical tests

Numerous attempts have been made to discover physico-chemical measurements by which it would be possible to predict the cold or drought resistance of plants. Measurements of bound water and sugars have perhaps received the greatest attention. The evidence obtained has been conflicting.

Clippings from a number of the most and the least cold- and drought-hardy species of turf grasses were taken at several different dates and analyzed for moisture, bound water, total nitrogen, and total sugars (table VII). From previous tests (**7**) it was known that with the approach of cold weather, there was an elaboration of hydrophilic colloids in *P. pratensis* and an increase in the total sugar content, accompanied by increased hardiness to cold, but at that time no comparison was made among different species.

In tests made in August and October, there was a lack of correlation between the bound water content of the different species and their demonstrated survival, or lack of survival, when exposed to drought. This disparity is particularly noticeable when *F. rubra fallax* is contrasted with *C. cristatus*, *P. trivialis* and *P. nemoralis*. Within the same species, the high-

TABLE VII

DATA FROM PHYSICO-CHEMICAL ANALYSES OF CERTAIN TURF GRASSES

GRASS	SECTION	GRAMS PER 100 GRAMS OF FRESH TISSUE									
		AUGUST		OCTOBER		NOVEMBER		DECEMBER		OCTOBER	NOVEMBER
		TOTAL MOISTURE	BOUND WATER	TOTAL MOISTURE	BOUND WATER	TOTAL MOISTURE	BOUND WATER	TOTAL MOISTURE	BOUND WATER	TOTAL SUGARS	PERCENTAGE OF NITROGEN IN DRIED TISSUE
		gm.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	%
P. pratensis	Low N	72.4	7.42	71.6	7.92	73.5	9.68	64.1	9.56	5.40	2.52
	High N	76.2	7.04	75.3	7.64	72.3	7.28	67.0	7.36	4.97	3.94
P. trivialis	Low N	73.8	6.32	75.4	6.72	77.3	6.38	63.0	7.48	4.42	2.98
	High N	77.0	6.48	81.0	5.86	79.1	5.26	76.4	5.74	4.01	3.86
P. nemoralis	Low N	73.2	7.26	75.3	7.58	75.8	7.42	66.0	9.96	4.15	2.44
	High N	75.8	.....	76.8	6.86	79.1	6.16	68.6	7.02	3.74	3.72
A. tenuis (Astoria)	Low N	71.8	7.12	73.8	7.71	71.9	9.18	67.5	9.16	5.17	2.70
	High N	76.6	6.98	76.0	6.41	74.3	7.04	71.2	7.84	4.17	4.23
A. canina (Highland)	Low N	72.7	6.86	73.0	7.13	72.4	8.76	66.8	9.43	4.73	2.61
	High N	76.2	.....	75.9	6.54	75.7	7.00	70.9	8.13	4.06	4.03
F. rubra fallax	Low N	69.8	6.38	74.4	6.82	77.3	7.30	66.9	9.46	4.12	2.07
	High N	74.2	.....	76.5	5.08	79.3	6.36	67.3	7.63	3.81	3.70
C. cristatus	Low N	71.3	6.48	75.0	7.08	72.0	7.90	62.6	9.36	4.53	2.16
	High N	75.8	6.62	80.4	5.14	82.0	5.82	64.2	7.18	3.92	3.44

nitrogen grass generally contained less bound water than the low-nitrogen grass and was always less resistant to drought. It should be noted that weather conditions previous to these tests were not conducive to hardening to drought. In tests conducted on *P. pratensis* in 1930 and 1931, however, when weather conditions favorable to hardening to drought prevailed, CARROLL and WELTON (7) found a lack of correlation between bound water content and drought hardiness in low- and high-nitrogen grass.

In November and December tests, which were made after the grasses had hardened to cold, there was a greater increase of bound water content in the low-nitrogen grasses than in the high-nitrogen grasses, and the low-nitrogen grasses possessed greater hardiness to cold. The differences in bound water content among a number of the grasses from the low-nitrogen section were negligible, but there were wide differences among them with respect to cold hardiness. Here, again, it must be said that on the basis of these tests, bound water cannot be accepted as a reliable index to the cold resistance of grasses.

Summary

A comparison was made of the survival of 15 species, representing 6 genera, of turf grasses exposed to soil drought and to extremes in soil and air temperatures. The effect of nitrogenous fertilizers in conjunction with these conditions was also investigated.

The term, soil drought, as used in this report, refers to conditions in the soil as it slowly dried to a moisture content of 5 per cent. or of 3 per cent., which is from 2.7 per cent. to 4.7 per cent. below the moisture content found to be the wilting coefficient of the Wooster silt loam on which the grasses were grown. Of the species tested, *P. trivialis, P. nemoralis,* and *C. cristatus* were most injured by soil drought. Those least injured were *P. pratensis, F. rubra fallax, F. rubra, A. tenuis* (Astoria and Cocoos species), and *A. canina* (Highland). The species from the high-nitrogen section were less able to withstand soil drought than the same species from the low-nitrogen section.

The species were exposed to an air temperature of −25° C. until soil temperatures of −5°, −10°, −15°, and −20° C. were reached. The species most injured by low soil temperatures were *L. perenne, L. multiflorum, C. cristatus, A. odoratum,* and *A. canina* (Highland). Those least injured were *P. pratensis, P. nemoralis, A. tenuis* (Cocoos), South German mixed bent, and *F. rubra fallax.* The lethal soil temperature for the majority of the species appeared to be between −10° and −15° C. The species from the high-nitrogen section were much less resistant to cold than those from the low-nitrogen section. Low soil temperature was more injurious than low air temperature.

Exposure to soil and air temperatures of 40°, 50°, and 60° C. showed that high soil temperatures were more destructive than similar air temperatures. The injury caused by high temperature of both soil and air appeared to be a direct thermal effect upon the protoplasm. The lethal temperature

varied with the time of exposure and for most species appeared to be between 50° and 60° C. Species from the high-nitrogen section suffered greater injury than those from the low-nitrogen section.

The data from tests made of bound water and sugars showed that the accumulation of these two constituents was decreased by the application of a nitrogenous fertilizer. These data were found to be unreliable as criteria of the relative hardiness of turf species to heat, cold, or drought.

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The Ohio Agricultural Experiment Station
Wooster, Ohio

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EFFECTS OF CERTAIN GROWTH REGULATING SUBSTANCES ON GROWTH CORRELATION IN LETTUCE SEEDLINGS

RICHARD B. STEPHENSON

(WITH NINE FIGURES)

The cultivation of excised plant parts in tissue culture has been used as one of the means of studying the effects of growth substances on the development of certain tissues. Wide use of this technique has been made in studying the growth requirements of excised roots (**6, 7, 8, 10, 12, 13**). Other embryonic tissues (**5, 9, 11**) and callous tissue (**14**) also have been cultured in this way. Full utilization of this technique, however, has not been made for the study of the effects exerted by the different organs of the plant on one another, as well as for the influences exerted by the different growth regulating substances upon the organ.

The present experiments were planned with the belief that something could be learned about the effects of growth regulating substances on the development of excised parts, and also about certain correlations within the plant by aseptically culturing various parts of the plant separately and in association with one another. Consequently, in many of the experiments four series of cultures were made: (1) whole plants, (2) excised roots, (3) excised shoots, and (4) excised roots together with excised shoots in the same flask.

Since the especial purpose of the investigation was to observe the effect of growth regulating substances on the early development of the plant, and to locate as nearly as possible the organs affected by these substances, no attempt was made to find an optimum basic medium for any one organ or for the plant as a whole. The medium used was considered satisfactory, since better growth resulted for the whole plant in the basic medium over the two week period the cultures were grown than in soil or sand culture.

Materials and methods

Seeds of the Grand Rapids variety of tip burn resistant lettuce were first soaked for two hours in a saturated solution of Semesan and were then passed through a 5 per cent. solution of bromine water and transferred to sterile, water-soaked paper in sterile Petri dishes. All operations were carried out in a transfer chamber previously washed with a 4 per cent. solution of formalin. The seeds were allowed to germinate for forty-eight hours before transfers were made to the culture solution. The roots were then ten to twenty mm. long, and the shoot, including the cotyledons, about the same length. For the series of excised roots, the terminal ten mm. were severed from the plant with a Bard-Parker scalpel and transferred to the culture solution on the tip of the scalpel. With a little practice this maneuver could be carried out successfully and the possibility of injury with forceps avoided. The scalpel was flamed with 95 per cent. alcohol before each operation, and

the necks of the culture flasks also were flamed. Contamination was infrequent. Shoots excised about 2 mm. below the cotyledonary node, as well as whole plants, were similarly transferred at this stage of development. A standard WHITE's formula of salts (**12**) with 2 per cent. sucrose was used in making up the solution to which the growth substances were added. Water twice distilled in Pyrex was used in making up all solutions, as well as for the final washing of the flasks. Fifty ml. of solution were added to each 125-ml. Pyrex culture flask.

All four series (excised roots separately, excised shoots separately, excised roots and shoots in the same flask, and intact plants) were cultured in each of the following concentrations of any particular growth substance: 1.0, 0.1, and 0.01 mg. per liter of the culture solution. Intact plants and excised roots were cultured also in a concentration of 10 mg. per liter, which in most cases caused great inhibition. Ten cultures were made of each series in each concentration. Measurements of the pH were made of each flask as a matter of routine after the tissues were removed. The data presented in table I show that the more actively growing tissues had induced a more alkaline reaction.

TABLE I

THE INFLUENCE OF THE RATE OF GROWTH UPON THE pH OF THE NUTRIENT SOLUTION, WHEN PLANTS WERE GROWN IN THE SOLUTION CONTAINING 0.1 MILLIGRAM OF THIAMIN PER LITER

ROOT GROWTH	pH
cm.	
16.4	5.52
21.8	5.65
22.1	5.87
25.9	5.90
26.1	5.99
26.9	6.00
30.8	6.20

Two criteria of growth were used in all cases: the length of root attained, and the dry weight of both the roots and the shoot. The comparison of the length and the weight serves as an index for the type of root growth (*i.e.*, long thin, short thick, etc.) taking place. The cultures were grown in daylight at room temperature ranging from 20° to 25° C. for a period of two weeks.

Results

The observations on these sets of experiments may be grouped into the following classes: (1) those which show the effects of leaving available to the plant, or of blocking off, the various possible paths for the transmission of correlative factors; and (2) those which show the effects of the different growth regulating substances tested. The factors correlating growth in the young lettuce seedling might be divided into two groups: those which could travel only through the living tissue of the plant; and those which could also

diffuse through a culture medium. It was for this reason that the excised parts were cultured together in the same medium, as well as in separate flasks. If histograms are made of the five series considered in class 1 for not only the controls but for each of the growth substances tested as well, then a simultaneous comparison of the effects from separating the plant parts and from the physiological effects of the chemicals may be made.

Correlation

Each histogram shows the mean of the total root growth per plant and represents the mean of at least ten cultures. Figure 1 represents the correlation group occurring in the basic medium; *i.e.*, salts and sucrose without added growth substance. The growth of the excised root alone, which is accountable only to those factors which were contained in the tip when it was excised, those which were manufactured by the root, and to the material of the culture medium, is the least of the five root types. The root which was cultured in the presence of the excised shoot, and which could be additionally influenced by substances diffusing from the growing shoot into the medium, grew slightly better. This effect, though slight, was very constant. The total root growth on the intact plants, including the main tap root, branch, and adventitious roots, is greater than that of any excised roots.

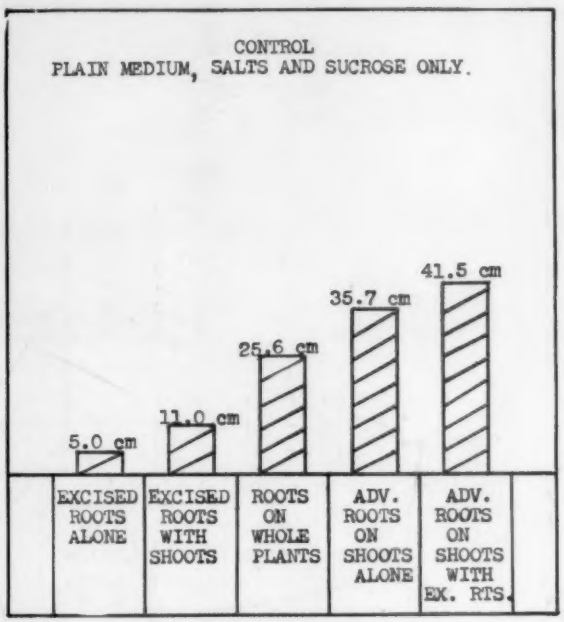

Fig. 1. Total mean root growth per plant in plain nutrient medium in the different correlation series. Excised root growth was slightly benefited by the presence of the shoot. Adventitious root growth on excised shoots exceeded total root growth on the intact plants. A further slight increase in adventitious root growth occurred when the excised root was present in the medium.

This growth could be influenced by factors produced in the shoot, and also by those in the growing tap root. That the latter is true is suggested by the growth of adventitious roots on excised shoots, which apparently are free from the restraining influence of some inhibitory substance associated with the main tap root. If the excised root is present in the same culture with the excised shoot, a further increase in adventitious root growth on the shoot occurs. This might indicate that the main tap root also supplies some substance stimulatory to root growth which may diffuse through the culture medium, and also that the inhibitory factor must pass through the living tissue of the plant. It is also possible that effects ascribed to the necessity

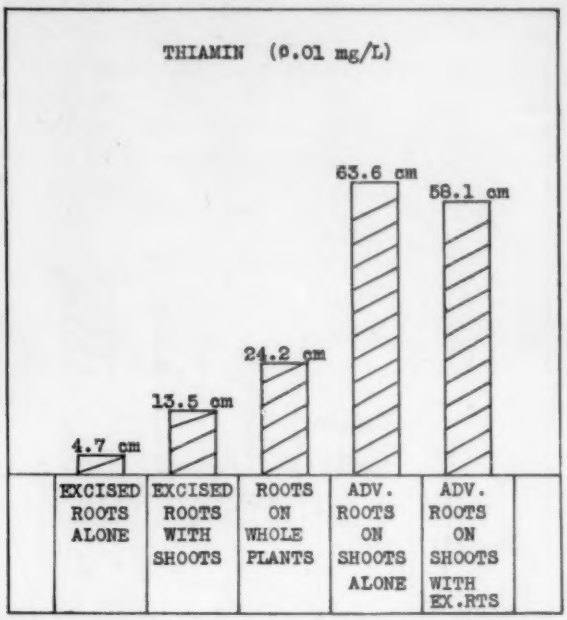

FIG. 2. Total mean root growth per plant with thiamin added to culture medium. The further stimulation of adventitious root growth on the excised shoots by the presence of the excised root as seen in the control series (fig. 1) did not take place.

for factors to pass through living tissue are actually due to the great dilution any substance must undergo while diffusing from any part of the plant through the culture medium.

Possible clues as to the nature of the factors, presumably "growth substances" of some sort, effecting these correlative differences, may be obtained from the histograms showing the growth that occurs when the different sets are grown in media to which the growth substances have been added. The substances used in these experiments were chosen at random from a large number of physiologically active substances which might have been used. No suggestion is made whatever that any of these actually take part in normal correlative activities within the plant, but it is pointed out that some of their effects are qualitatively similar to those obtained when the plant's normal paths of correlation are broken.

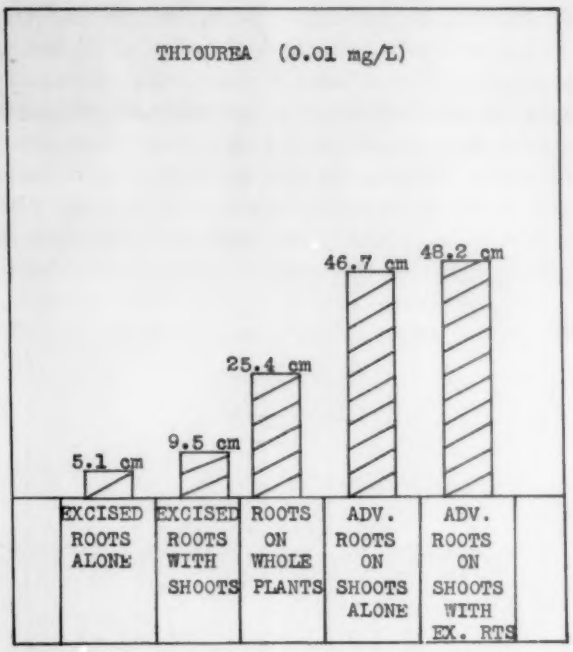

FIG. 3. Total mean root growth per plant with thiourea added to culture medium. This histogram does not appear to be significantly different from that for the control series (fig. 1).

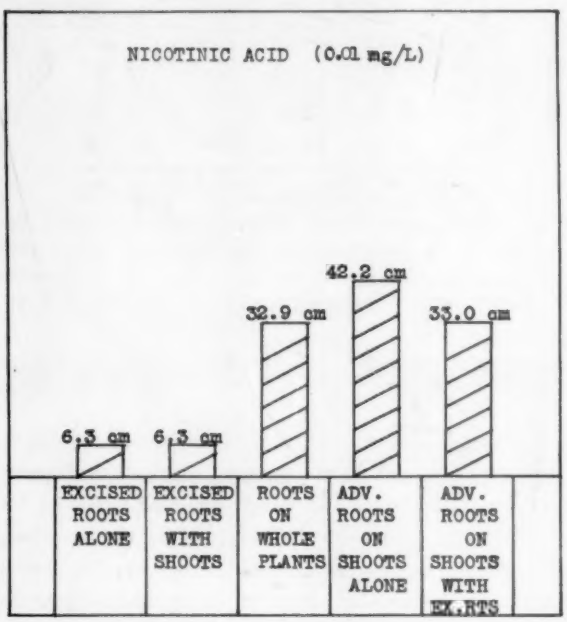

FIG. 4. Total mean root growth per plant with nicotinic acid added to culture medium. This figure is similar to that for thiamin (fig. 2) with the further difference from the control that there was no stimulation of the excised root by the shoot.

When thiourea (fig. 3) was added to the culture medium, the general trend of the histogram was not changed from that of the basic medium, and hence its rôle, if any, in correlation is not shown. Thiamin (fig. 2) does not greatly alter the shape of the figure, except that no increase in adventitious root growth on shoots cultured with excised roots occurs over that in those cultured alone. This is also true of nicotinic acid (fig. 4), in which, in addition, the characteristic slight increase in growth (for the control) of the excised root taking place in the presence of the shoot did not occur. Accordingly, it appears that the usual reciprocal relationship between the

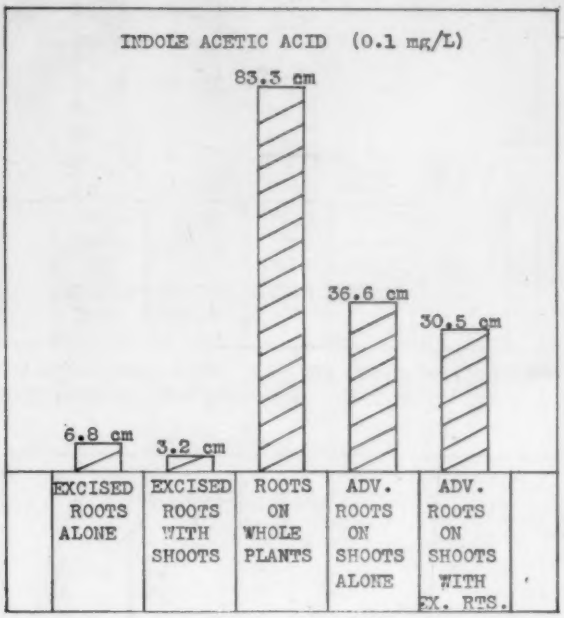

FIG. 5. Total mean root growth per plant with indole acetic acid added to culture medium. Here the growth of the excised roots alone was definitely greater than when in the presence of the shoot. The increased growth of roots on the intact seedling overshadowed the response of the adventitious roots both alone and in the presence of the root. These responses and relationships were all exactly the opposite of those observed in the control series.

root and the shoot in the control group is altered by thiamin and nicotinic acid.

The figures for indole acetic acid (fig. 5) and naphthalene acetic acid (fig. 6) are very similar, except for magnitude. The decreased growth of the excised root cultured with the shoot, as compared with that cultured alone, suggests that the substance diffusing from the shoot may be physiologically similar to these substances—that is, an auxin, since other experiments have shown that these will cause an increase in the growth of excised roots if the concentration is low enough, but are inhibitory in higher concentrations. The reversal in trend of the other parts of the figure may be due

in part to the large number of adventitious roots which are induced on the whole plant, and for which some interaction of substances conveyed through living tissues from both the main root and shoot seems to be necessary. This appears reasonable since the number of adventitious roots developing on excised shoots is less when auxins are present in the medium and also because those lateral roots which are induced on excised roots are very poorly organized. In high concentrations (ten milligrams per liter) of these substances, adventitious roots fail to develop at all.

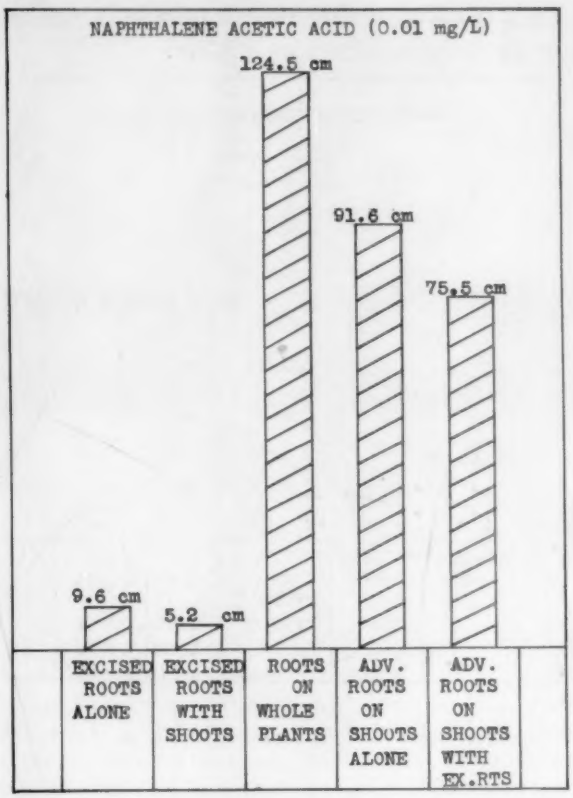

FIG. 6. Total mean root growth per plant with naphthalene acetic acid added to culture medium. The relationships seen here were like those in indole acetic acid, but with a wider latitude of response.

That naphthalene acetamide (fig. 7) also causes an increase in excised root growth, and that the growth of the root alone is greater than when it is with the shoot seems to indicate that in some cases the auxins with the free acid group may themselves be the acting agent in the plant. The rest of the group for naphthalene acetamide shows the trend typical of the basic medium rather than that typical of the auxins. This indicates that in some cases others of the diverse effects of these substances may occur through the formation of secondary compounds, such as the amide linkage.

Growth substances

The effects of the growth substances as compared with the controls are comprehensively shown in table II, which is complete for the two most extensively studied concentrations, 0.1 and 0.01 mg. per liter.

Many reports have been made of the effect of thiamin on plant growth. None of our findings indicate that it has a stimulatory effect on the growth of intact lettuce plants. This concurs with the results of Bonner and Greene (3) with tomato and some other plants. These authors assume that most of the annual green plants are able to produce enough thiamin so that it does not limit root growth in the normal plant.

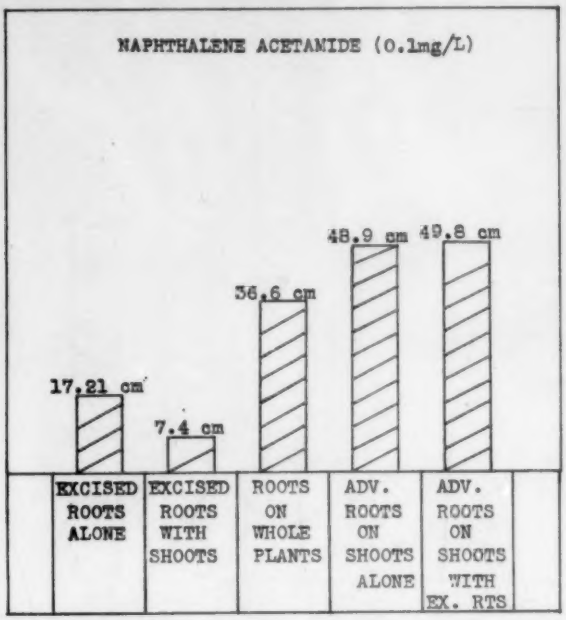

Fig. 7. Total mean root growth per plant with naphthalene acetamide added to culture medium. The excised roots showed a response similar to that found in naphthalene acetic acid and indole acetic acid, but root growth on the shoot and the intact plant was not greatly different from the control. This split response should be especially noted.

A concentration of ten mg. per liter of thiourea produced a slight increase in root growth and in the dry weight of the plant. This high concentration may be effective partly because of the mere added supply of nitrogen as a nutrient, but this does not seem probable. In other respects the results obtained with thiamin and thiourea were similar. In concentrations of 0.1 and 0.01 mg. per liter a consistent and comparable stimulation of adventitious root growth by both substances occurred on excised shoots. This was not always accompanied by an increase in dry weight, as these roots were very long, infrequently branched, and exceedingly small in diameter. It should be noted that the adventitious roots appear on the shoots in most cases within 24 hours after their transfer to the culture flask.

TABLE II

SHOWING THE EFFECTS OF THE VARIOUS SUBSTANCES ON GROWTH IN THE DIFFERENT SERIES. MEASUREMENTS EXPRESSED AS PERCENTAGE OF CONTROL MEAN FOR THAT SERIES

SUBSTANCE	CONCENTRATION PER LITER	EXCISED ROOTS ALONE		EXCISED ROOTS WITH SHOOTS		WHOLE PLANTS			SHOOTS ALONE			SHOOTS WITH ROOTS		
		LENGTH	DRY WEIGHT	ROOT LENGTH	DRY WEIGHT	ROOT LENGTH	DRY WEIGHT OF ROOT	DRY WEIGHT OF SHOOT	ROOT LENGTH	DRY WEIGHT OF ROOT	DRY WEIGHT OF SHOOT	ROOT LENGTH	DRY WEIGHT OF ROOT	DRY WEIGHT OF SHOOT
	mg.	%	%	%	%	%	%	%	%	%	%	%	%	%
Thiamin	0.10	119.6	94.7	130.5	176.2	88.6	74.4	105.0	160.7	170.3	102.1	135.7	105.6	115.2
	0.01	94.0	76.3	123.2	154.0	94.4	71.6	81.0	178.3	220.0	162.7	140.0	152.9	136.9
Thiourea	0.10	117.6	89.5	114.2	116.0	84.0	65.2	81.0	178.9	271.7	180.2	117.8	97.1	82.3
	0.01	101.0	26.7	86.7	128.6	98.9	100.4	74.8	131.0	106.7	88.4	116.0	110.0	110.1
Nicotinic acid	0.10	159.2	81.6	159.7	196.8	99.5	91.2	62.6	104.4	80.0	55.2	139.7	141.6	121.5
	0.01	125.0	139.0	57.8	71.4	128.4	147.0	102.7	118.3	95.0	70.8	79.5	85.7	79.3
Naphthalene acetamide	0.10	376.0	271.1	67.5	54.0	142.7	107.8	117.8	137.3	166.7	183.0	110.4	158.7	113.7
	0.01	177.0	560.5	44.2	95.7	138.0	135.0	93.8	87.3	96.7	98.8	86.4	136.7	103.0
Naphthalene acetic acid	0.10	.....	286.8	.....	120.6	.....	681.0	50.0	.....	840.0	58.3	.....	790.0	95.2
	0.01	193.2	1210.5	47.0	351.0	458.8	1673.2	257.8	257.0	1178.3	232.1	181.8	565.6	246.9
Indole acetic acid	0.10	135.8	560.5	28.7	63.5	324.7	669.3	249.6	102.8	451.7	177.8	75.3	128.6	105.5
	0.01	332.2	765.8	58.0	47.6	104.8	274.0	73.6	123.5	583.3	233.7	108.9	138.6	122.3

Nicotinic acid also has been reported to be required for the continued growth of excised roots (1) and to stimulate the growth of certain plants (2). Its effect on lettuce is similar to that of thiamin and of thiourea, but was toxic in concentrations of 10 and 1 mg. per liter. A concentration of 0.01 mg. per liter produced definite stimulation of the growth of excised roots.

The inhibitory effect of auxins on root growth has been repeatedly reported in the literature although certain workers have found a stimulation. This apparent discrepancy is perhaps due to the variation of experimental method, as well as to the variations in response of different species.

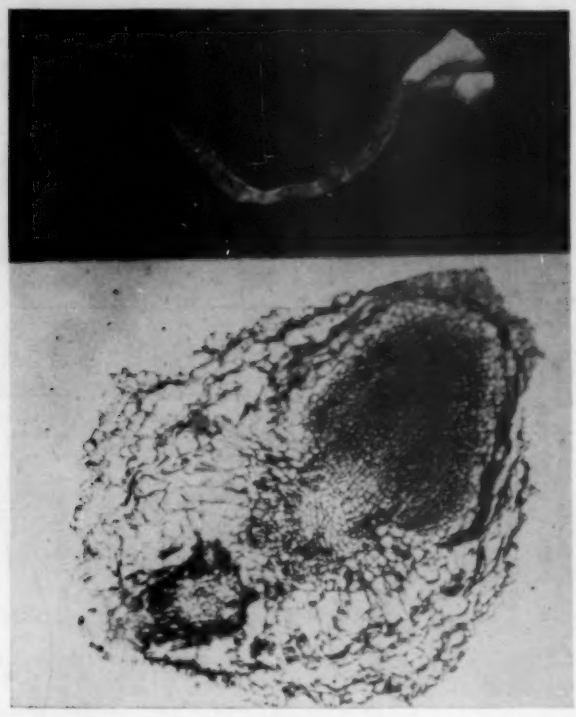

FIG. 8. Whole plant cultured in nutrient plus ten milligrams per liter of naphthalene acetic acid, showing nodule-like inhibited root primordia, and below, transverse section through one of them.

GEIGER-HUBER and BURLET (8) and FIEDLER (7) report increase of growth for the lower concentration under conditions similar to our experiments; BONNER and KOEPFLI, however, (4) report inhibition of root growth in Avena for the same concentration in which the greatest growth took place in these experiments. Their experiments, however, lasted only over a 24-hour period. It is possible that the stimulation which was observed here is due to a secondary effect since the culture solution at the end of the two weeks does not give curvature in the Avena test. The auxin is therefore presumably either used up or in some way destroyed by the plant, but the

fact remains that the stimulation of growth is the result of addition of the auxin to the culture.

The auxins used in these experiments, indole acetic acid and naphthalene acetic acid, gave very similar results. The results, however, with naphthalene acetic acid were roughly comparable to those in ten times the concentration of indole acetic acid. No normal growth occurred in the ten-mg.-per-liter concentration of either auxin. Excised roots were completely inhibited in their growth and no nodular growths (inhibited primordia) were formed. The entire plant elongated but a few millimeters in this concentration. Thickening of the leaves occurred, and many nodular growths were formed

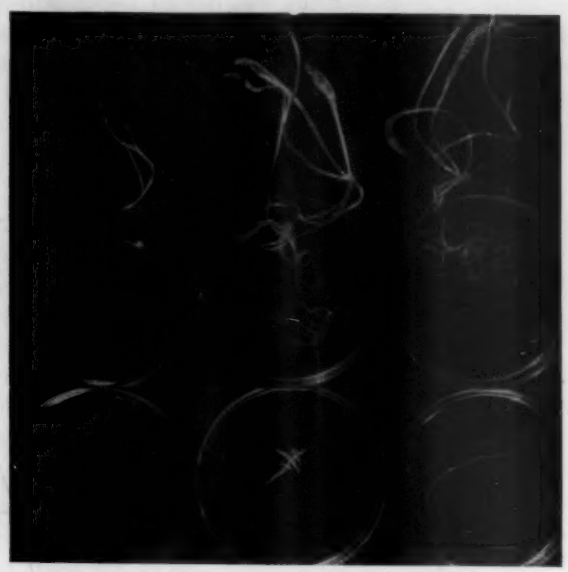

FIG. 9. Above: left, whole plant in nutrient only; center, plus 0.1 mg. per liter indole acetic acid; right, plus 0.01 mg. per liter naphthalene acetic acid. Below: left, upper, excised root in nutrient only; lower, plus 0.1 mg. per liter thiamin; center, plus 0.1 mg. per liter indole acetic acid; right, plus 0.1 mg. per liter naphthalene acetamide.

on both the root and the epicotyl (fig. 8). These nodules are evidently the result of the initiation of root primordia which are subsequently inhibited in their growth.

In the 1.0-mg.-per-liter concentration of auxins almost no growth takes place, but some primordia are initiated on the excised roots as well as on the shoots and on the intact plants. Those on the excised roots, however, are poorly organized and often resemble a cancerous growth more than root primordia. More primordia were initiated in naphthalene acetic acid than in indole acetic acid and subsequent inhibition was greater, resulting again in a nodule-like appearance as in the 10-mg.-per-liter concentration. The primordia on the shoot and on the intact plant developed somewhat giving rise to very short, thick, and profusely branched roots. No branch roots

developed on the excised roots. The comparative counts of numbers of primordia and lateral roots showed that there are more primordia formed on separate shoots than on shoots when the root is present; but the number on the roots is greater in the latter case than on the roots cultured separately, and also the number on shoots alone is greater than the sum of those initiated on both the roots and shoots cultured together.

Great stimulation of root growth occurred in all series in the 0.1- and 0.01-mg.-per-liter concentrations of both auxins (fig. 9). The length of root was not determined in the 0.1-mg.-per-liter concentration series of naphthalene acetic acid since there were a very great number of exceedingly short, thick roots whose linear measurement was almost impossible to determine, and, even then, would have been without significance. Swelling of the cortex also occurs and is occasionally so severe that the outer layers of the cortex are completely separated from the central cylinder. Roots were in all cases much thicker than those of control plants.

Summary

In an effort to approach simultaneously the problems of growth correlation and the effects which certain reported growth regulatory substances exert upon young seedlings, the author utilized aseptic tissue culture technique methods to compare four series of cultures: (1) intact seedlings, (2) excised roots, (3) excised shoots, and (4) a combination of excised roots and excised shoots. In these four series, thiamin, thiourea, nicotinic acid, naphthalene acetamide, naphthalene acetic acid, and indole acetic acid were added to the culture medium of inorganic salts and sucrose in concentrations from 0.01 to 10 mg. per liter. The control group (without added substances) showed that excised root growth was slightly benefited by the presence of the shoot in the medium, that the growth of adventitious roots on the excised shoot normally far exceeded that of the roots on the intact seedling, and that a further slight stimulation of this growth of adventitious roots on the excised shoot occurred when the excised root was present in the medium. When this group was compared with those obtained for the various growth substances in the lower concentrations, certain striking differences were seen. In thiamin the further stimulation by the excised root of the adventitious root growth on the excised shoot did not occur. Neither did it occur in nicotinic acid. Also the characteristic slight increase in growth of the excised root taking place in the presence of the shoot did not occur in nicotinic acid. It appeared that both of these substances, then, disturbed the usual reciprocal relationship between the root and the shoot as found in the control group. Thiourea did not appear to alter this relationship, which was affected to a greater extent, although differently, by naphthalene acetic acid. Naphthalene acetic acid brought about an increase in the growth of excised roots alone, both over the control, and over those also in the naphthalene acetic acid, but in the presence of the shoot. This is directly opposed to the relationship of the two excised root series in the

control group. Similarly, the increase in root growth on the intact seedling brought about by naphthalene acetic acid overshadowed the response of the adventitious roots on the excised shoot, which in turn was greater when the shoot was alone than when it was in the presence of the excised root. This again was exactly the reverse of the situation in the control group. The same type of response was noted with indole acetic acid. It was also found in the response of excised roots to naphthalene acetamide, but the growth of roots on all three series of shoots in this substance followed the trend of the control group. The striking difference between the response of the different parts of the plant to the acid and the amide of the same substance should be noted.

Various morphological irregularities were observed in the higher concentrations of some of the substances.

The author believes that problems concerned with the effects of growth regulating substances on the plant must be attacked simultaneously with those on growth correlation, or any conclusions derived therefrom will be seriously limited in their scope.

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University of Illinois
Urbana, Illinois

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EFFECT OF THE ROOT SYSTEM ON TOMATO STEM GROWTH

F. W. WENT

(WITH TWO FIGURES)

A long succession of observers have found a correlation between elongation of internodes of the stem axis and the presence of the stem tip. Botanists are in general agreement at present that this correlation may be attributed to the formation of auxin in the stem tip and its transmission through the stem to the elongating regions. Shoot growth is also dependent upon factors supplied by the root system. Vigorously growing branches soon suspend growth in length after they have been cut and placed in a vase. This root influence on shoot elongation is not due to the better known functions of the root system, namely water and salt uptake, for no set of conditions insuring adequate water and salt supply of the cut shoot can replace the loss of the root systems. In several papers (**14, 15, 17**) this effect of the root system on growth has been investigated in some detail and definite indications of the substantial nature of this effect have been presented. An analysis of the distribution of growth rates in the Avena coleoptile also led to the assumption of a second growth factor in addition to auxin required for stem elongation. In earlier papers this factor was non-committally named "food factor" (**12, 13**), but later when it became evident that sugar was not identical with this "food factor" (**9, 10, 17**) a special name, caulocaline, was used for this second factor without any commitments as to its nature.

In experiments with pea seedlings the root system was found to exert its specific effect on shoot growth even when it did not have to take up nutrients, and the effect was most pronounced when the roots were in contact with the minimum amount of water. If the roots were submerged too far in the non-aerated tap water, shoot growth was much decreased (**15, 17**). BONNER and AXTMAN (**3**) and SKOOG (**11**) found that in excised embryos the presence of growing roots increased shoot growth. This is remarkable because one would rather expect the shoots to be in food competition with the roots.

All this evidence leads us to the hypothesis that under proper conditions the root system produces a hormone, caulocaline, which is required for stem growth in conjunction with auxin and sugar (**17**). In the present paper the conditions under which the root system exerts its influence on stem growth was studied. All work was done with tomatoes grown in the greenhouse.

Methods and results

To analyze the different functions of the root system, young tomato plants, San José Canner variety, were grown in sand. When they had reached a length of 10 to 15 cm. the root system was washed free of the adhering sand, and the stem below the cotyledons was split lengthwise so that on one

plant two separate root systems were obtained, each attached to one half of the stem base. The plants were then placed over two adjoining containers with HOAGLAND nutrient solution (**6**) so that half of the root system dipped into each container. The nutrient solution in each container was aerated, and within two weeks the root systems were well developed. Then the two halves of the root system could be subjected to different conditions in an attempt to separate its various functions. As an example, one of the first experiments will be described.

The plants were divided into three groups of 5 to 10 plants each. Group A remained with both portions of the root systems in nutrient solution. Group B consisted of plants in which one-half of the root system was killed, so that they had only one-half of the functional root system in the culture solution. In group C the nutrient solution around the one-half root system was left, but around the other half it was exchanged for peat, which was kept moist with tap water.

Figure 1 indicates the rate of stem elongation of the three groups. Be-

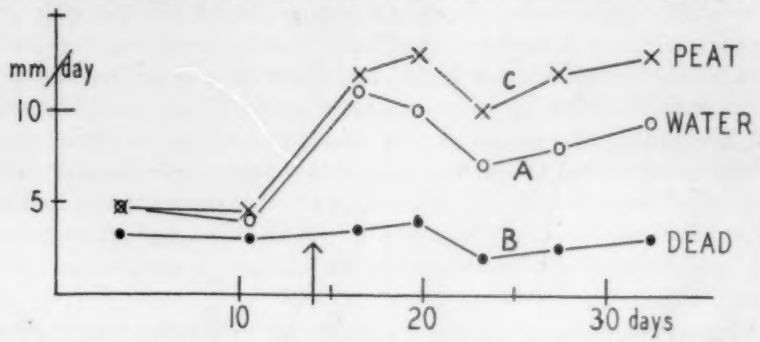

FIG. 1. Growth rate (ordinate, mm./day) of stems of tomato plants with split stem bases and root systems. Both halved root systems of each plant are submerged in nutrient solution, until the 14th day (arrow), when one half is left in the solution, and the other half is either transferred to peat (C), or is left in solution (A), or is dead (B).

fore transfer of roots of C, groups A and C had the same growth rate. In those plants the root system was already close to limiting the growth rate, for plants with only one-half living root system grew less (group B).

Four days after transfer of group C its growth rate was still approximately the same as that of A, but in subsequent periods the growth of the former became and remained significantly above that of A in spite of the fact that the root system effective in taking up salts and water was reduced to one-half. The increase in growth rate followed the appearance of many new roots with abundant root hairs on the root system in peat. Observation showed that relatively little water was taken up from the peat, the bulk coming from the nutrient solution.

In this and later experiments it was noted that even with abundant aeration of the nutrient solution, chlorosis developed in the tomato plants having all roots submerged in the solution. This chlorosis became especially

severe when the pH of the nutrient solution was approximately 7, and it was less pronounced at pH 5 to 6; even at this lower pH the plants were only light green. Even the severest chlorosis disappeared, however, as soon as roots developed in the peat, or above the nutrient solution. This might have been due to improved uptake of iron from the peat, since iron humates are known to be present in peat and to be an excellent source of iron for the plant. For this reason, an inorganic inert medium was compared with peat. For this inorganic medium $\frac{1}{8}$- to $\frac{1}{4}$-inch-mesh haydite was chosen, a pumice-like, light-weight, inert, burned-shale, sharp-edged, material which can hold a considerable amount of water and gives aeration as good or better than peat. Four groups of 5 tomato plants each were set up with halved root systems. The growth rate of the groups was comparable and almost constant for a period of two weeks as shown in table I. After transfer of one-half the root system to the solid medium, the growth rate of these plants almost doubled one week after the transfer whereas the growth rate of the plants with both root systems in solution fell off to a very low rate.

This same type of experiment was repeated at least ten times, always with the same results. When the pH of the nutrient solution was kept at 5, the growth of the plants with both root systems in solution was better than at a higher pH, but it was always exceeded by the plants with one portion of their root systems not submerged in solution. This fact is stressed by the experiment shown in table II, where the growth rate of the plants with one portion of the root system in silica gravel failed to increase above that of the control plants. This was due to the fact that for the first 13 days after transfer to gravel the water level was kept up to the surface of the gravel. Upon draining of the gravel the growth rate immediately increased. In this case, a pure quartz gravel washed for 5 hours with strong H_2SO_4, then leached with rain water for 24 hours was used. Its color did not indicate the presence of any iron. Still the plants with half of their roots in this material, watered with rain water, became dark green. When half of the root system of these plants was cut off, only those plants having roots left in the gravel continued to develop green leaves even though they had no roots left in the nutrient solution. All of these experiments show that the effect of roots growing outside the nutrient solution upon the formation of the green color of the leaves is neither through iron uptake nor the iron uptake of the other roots, but by making iron (and other elements) available for chlorophyll formation. These roots can even offset the bad effect of high pH in the nutrient solution.

A summary of the data on the growth of the stem from 7 experiments, involving 140 plants, and all giving the same qualitative results, is presented in table III. The growth rate of the control plants remained constant or dropped over a 25-day growing period. The growth rate of the treated plants rose immediately after transfer of a portion of their root system to a solid moist medium.

The problem was also attacked with a slightly different technique. In-

TABLE I

GROWTH RATE IN MM./DAY OF TOMATO PLANTS (3 TO 5 PER GROUP) WITH SPLIT ROOT SYSTEM, THE HALVES SUBMERGED IN DIFFERENT CONTAINERS WITH NUTRIENT SOLUTION*

GROUP	ROOTS IN CONTAINER NO.	LENGTH IN MM. MARCH 19, 1940	MARCH 19–25	MARCH 25 TO APRIL 2	APRIL 2, 1940 HALF ROOT SYSTEM IN NUTRIENT OTHER HALF IN	APRIL, 1940							LENGTH IN MM. APRIL 25, 1940
						2–5	5–8	8–11	11–15	15–18	18–22	22–25	
A	1 2	100	7.8	7.0	Peat	5.7	9.0	7.7	10.0	10.0	7.5	15.7	417
B	2 3	98	7.0	8.0	Nutrient	6.7	8.0	4.7	1.5	3.3	2.0	2.0	292
C	5 4	104	7.7	6.0	Haydite	6.7	7.7	14.7	15.0	12.0	12.5	14.7	497
D	4 3	104	6.0	6.5	Nutrient	6.7	9.3	6.7	3.0	1.7	1.2	0.7	294

* The second column shows that, *e.g.*, each of groups B and D had one-half of their root system in container 3.

TABLE II

ROOT SYSTEM OF TOMATO PLANTS SPLIT ON MARCH 18, 1941. BOTH HALVES WERE AT FIRST IN NUTRIENT SOLUTION WITH ADDED IRON; pH OF SOLUTIONS WAS KEPT BETWEEN 5.2 AND 6.0. ON MARCH 29 ONE HALF OF THE ROOT SYSTEM OF 10 PLANTS WAS TRANSFERRED TO SILICA GRAVEL. UNTIL APRIL 11 THE GRAVEL WAS COVERED WITH RAIN WATER, WHICH WAS DRAINED OFF ON THAT DATE, LEAVING THE GRAVEL MOIST, WITH EXCELLENT AERATION

ROOT SYSTEMS	GROUP	GROWTH RATE IN MM./DAY							
		MARCH, 1941			APRIL, 1941				
		21–28		28–3	3–11		11–14	14–18	18–21
		mm.		*mm.*	*mm.*		*mm.*	*mm.*	*mm.*
Both in nutrient solution	A	4.8		5.9	11.2		7.7	10.6	7.0
One in nutrient solution, the other transferred to gravel on March 29	B	5.0	One half root system in gravel	10.7	10.6	Water drained off gravel	10.7	13.1	8.3
Growth of group B in percentage of group A		104.0		95.0	95.0		139.0	124.0	119.0

TABLE III

A SUMMARY OF STEM GROWTH (FOR 3.5-DAY PERIODS IN MM./DAY) OF TOMATOES GROWN WITH BOTH PORTIONS OF THEIR HALVED ROOT SYSTEMS IN NUTRIENT SOLUTION (TOP ROW). MEAN OF SEVEN EXPERIMENTS, EACH COMPRISING 20 PLANTS

	OBSERVATION PERIOD IN DAYS						
	3.5	3.5	3.5	3.5	3.5	3.5	3.5
Both portions of the root system in nutrient*	8.8	8.5	8.0	8.8	9.0	6.3	6.4
One half root system in nutrient, other half transferred to solid medium after 3rd period	8.6	8.3	7.7	11.1	12.4	10.8	9.9
Growth of treated group in percentage of control group	98	98	97	129	138	170	154

* Figures in second row refer to plants 10.5 days before and 14 days after one portion of their root system was transferred from nutrient solution to either moist peat, haydite, silica gravel, or glass wool.

stead of mechanically dividing the root system into two parts, tomato plants were induced to develop a root system outside the nutrient solution in addition to the roots in the nutrient. This was done by growing tomatoes in wire baskets containing a layer of about 3 cm. of peat, haydite, gravel, sand, or soil, which was kept wet with tap water. These baskets were suspended

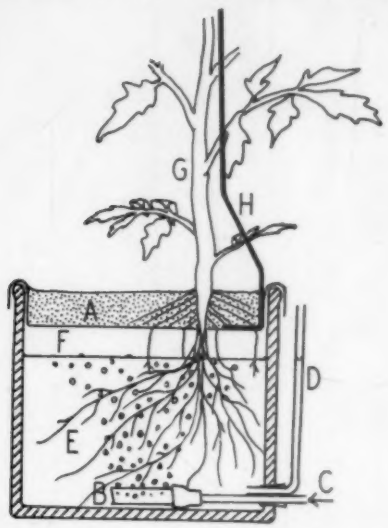

FIG. 2. Cross section through a two gallon earthenware crock, two-thirds filled with nutrient solution (E), through which air (C) is finely divided with aerator (B). The water level can be read at D. On top of the crock is attached a wire basket, filled with peat (A). In this peat a tomato plant (G), tied to support (H), develops a root crown, and in the solution the feeder roots branch out. F is air space between peat and nutrient solution.

from the edge of two-gallon crocks, two-thirds filled with nutrient solution, which was well aerated (fig. 2). The stems of tomatoes, germinated in sand and transplanted into the baskets, extended through the wire basket so that all roots dipped into the nutrient solution, and within two weeks from planting, a second root system developed in the medium in the basket. In general, those roots remained short and had many root hairs, but occasionally some of them grew down into the nutrient solution and then elongated very much.

Under these conditions the tomatoes grew slowly until the roots in the basket were well developed, then their growth increased to approximately the same rate as that of tomatoes grown in sand and watered with nutrient. The following growth rates in mm. per day were measured for plants approximately 300 mm. tall in a humid greenhouse (70 to 80 per cent. humidity, 26.5° C.); 14.6 with haydite in basket; 14.3 with peat in basket; and 16.1 in ordinary sand culture. In the dry greenhouse (30 to 40 per cent. humidity, 26.5° C.) 11.6 with haydite and 10.2 with peat. In other instances growth rates as high as 27.2 mm. per day were measured, which compared with 26.9 for similar tomatoes grown in gravel with sub-irrigation (both with day temperatures of 26.5° C. and night temperatures of 20° C.). The aeration of the solution is of no importance for the growth of the tomato plants as soon as roots have developed in the basket. In some experiments it was even found that aeration decreased the growth rate in direct proportion to the amount of air passed through the solution. In 16 non-aerated plants the growth rate was 11.1 mm. per day. With an air stream of 0.5 to 2.5 ml. per minute the growth rate was 9.0; from 16 to 17 ml. per minute it was 8.6; from 25 to 60 ml. per minute it was 8.4, and from 100 to 180 ml. per minute it was 7.1. In another experiment 18 tomato plants with their roots in an aerated nutrient solution grew at a rate of 22.9 ± 1.6 mm. per day over a two-week period, whereas 16 comparable plants, in unaerated solution, grew 26.3 ± 1.0 mm. per day. This same difference was maintained in following weeks. The standard deviation in the aerated plants was in every case higher than in non-aerated plants. This was due to a much greater number of plants with extreme growth rates, mainly with extremely low rates.

In the plants in the baskets chlorosis also developed when the pH of the culture solution was above 6 and when no roots had developed in the medium in the basket. As soon as the roots grew out in this medium chlorosis disappeared. Sometimes only half of the plant became green and in these cases it was found that roots had developed only at the side of the stem below the green sector. This localized effect was also observed in the tomatoes with the halved root system described before. Here also the sector of the tomato plant above the root system in the solid medium turned green and only much later the whole plant changed color. Another indication that the nutrient solution as such is not responsible for the chlorosis was found in the observation that two plants in the same basket with their roots

TABLE IV

GROWTH RATES IN MM./DAY OF PLANTS GROWING UNDER DIFFERENT CONDITIONS, AND THE GROWTH RATES OF THESE SAME PLANTS AFTER THE ROOTS DEVELOPED IN HAYDITE OR SOIL IN THE BASKET HAD BEEN CUT OFF

	Basket filled with	Growth rate in mm./day								
		Original length April 18	April				May			
			18–21	21–25	25–28	28–5	5–9	9–12	12–16	16–19
Solution aerated	haydite	24.1	5.2	7.9	10.5	16.0	20.7	19.7	18.7 11.2*	18.7 10.0
Solution not aerated	haydite	28.5	4.7	9.7	19.7	20.4	27.2	28.3	21.6	21.6
Solution not aerated	soil	25.0	5.7	9.6	24.1	24.1	29.2 27.0†	27.3 18.3	26.9 19.2	26.9 17.7

* Roots in basket cut off May 12.
† Roots in basket cut off May 7.

in the same solution might be very different. The one with roots in the peat, sand, gravel, or haydite was dark green and grew rapidly, whereas the one without a root system outside the nutrient solution was yellowish green and remained stunted.

That the growth rate of the plants in these baskets is in the first place determined by the roots developing in the medium above the culture solution was indicated by the fact that the growth rate of the plants remained low as long as the roots in the basket had not developed. The complementary experiment in which the roots growing in the basket were being cut off gave the expected result (table IV). In another experiment, within a week after cutting the roots in the basket, the growth rate of the tomatoes had dropped to one-third of that of the controls in spite of the fact that the dry weight of these roots, which were removed, was much less than 10 per cent. of the dry weight of all roots. By cutting off four-fifths of the root system which developed in the solution the growth rate temporarily dropped to about 50 per cent., but soon returned to normal. This indicates that less than ten per cent. of the root system in those tomatoes is responsible for more than 50 per cent. of their growth rate.

It would be expected from the previous experiments that there is a rather close correlation between the growth rate of the tomato stems and the weight of the root system developed in the basket. In an attempt to determine whether this was due to this root system as such or to other factors a number of determinations of the sugar content, auxin content, osmotic concentration of the cell sap, etc., were carried out in two sets of plants which had shown great difference in growth rate. One set of plants had been growing in a green house maintained day and night between 26° and 27.5° C. and 30 to 40 per cent. humidity; the other set was grown under exactly the same conditions except that the humidity was kept between 70 and 80 per cent. Under these conditions tomato plants grown in gravel with sub-irrigation showed exactly the same growth rate (for a weekly period in the dry house 21.5 mm. per day, in the wet house 21.5 mm. per day). The difference in growth rate of the two sets of plants grown in baskets above nutrient solutions, therefore, was not due to the different humidities as such. At the low humidity the peat in the basket dried out more rapidly and therefore had decreased the development of roots. In table V a number of the determined values have been condensed. These figures show a great difference in growth rate of the stems between the tomatoes grown in the dry and in the wet atmospheres. They also show that the difference is not correlated with sugar content of leaves, leaf development, root development in the nutrient solution, osmotic concentration and pH of the roots and stem tissues, auxin content of the green tips, or thiamin content of leaves, tips, and roots, for they all are of the same magnitude in the two groups of tomatoes with the different growth rates. Under wet conditions the stomata were more open, and the suction force was slightly less; but these same differences were found in the plants under sub-irrigation which did not show any differences in

growth rate. The only outstanding difference between the two sets of plants was the weight of the roots in the basket and stem length, weight, and growth.

The root system in some solid, well-aerated medium is not essential for growth. Even when the whole root system is completely submerged in nutrient solution growth takes place, although in the author's experiments at a decreased rate. If the iron content of the nutrient solution is sufficiently high (10 to 100 times more than required when used in sub-irrigation) and when the pH is carefully kept adjusted, chlorosis does not necessarily develop in tomatoes which have their complete root system submerged

TABLE V

VARIOUS VALUES FOR TOMATOES GROWN IN BASKETS ABOVE NUTRIENT SOLUTIONS AT THE SAME TEMPERATURE AND LIGHT CONDITIONS BUT IN DIFFERENT RELATIVE HUMIDITIES.* EACH VALUE IS THE MEAN OF 3 TO 8 DETERMINATIONS

	WET HOUSE	DRY HOUSE
Total length when harvested (mm.)	362	271
Growth rate in mm./day	24.7	13.8
Dry weight of leaves (mg.)	1518	1228
" " per leaf (mg.)	134	123
" " of roots in basket (mg.)	155	72
Wet weight of roots in solution (gm.)	6.75	6.47
" " " stems (gm.)	20.72	11.35
Osmotic concentration of press sap from roots (atm.)	4.82	4.46
" " " " " " stems (atm.)	8.19	8.07
pH of press sap from roots	5.52	5.50
" " " " " stems	5.20	5.20
Auxin content of tops in degrees curvature/gram	74	80
Vitamin B_1 in γ/gram dry weight of tops	14.3	12.0
" " " " " " " leaves	8.2	8.5
" " " " " " " roots in basket	6.8	6.5
Glucose; percentage dry weight of leaves	0.82	0.88
Sucrose " " " " "	0.61	0.79
Suction force (atm.)	8.71	11.1
Opening width of stomata (10 = wide open)	3.8	1.9

* Wet house, 75 per cent.; dry house, 35 per cent.

in nutrient solutions. A very small proportion of all roots if outside the nutrient solution and in a healthy condition both offsets unfavorable pH or low iron content of the nutrient solution and greatly increases the growth rate. Even roots which have developed in the saturated atmosphere above a nutrient solution can perform this function.

Only very few experiments were carried out to investigate whether the results obtained with tomatoes applied to other plants as well. With Cosmos very striking effects were observed. When young plants 8 cm. in length were transplanted in the peat baskets with their roots in the well-aerated nutrient solution, some growth occurred; but within 1 to 2 weeks the newly formed leaves were practically white, growth came to a standstill, and the completely etiolated tops started to die. In a few plants this condition im-

proved again, and in all such plants roots were found which had developed above the nutrient solution or in the peat. Increasing the iron and minor elements in the culture solution did not give the slightest improvement, whereas the same solution produced good growth when used to water Cosmos plants growing in sharp washed river sand or pure quartz sand or haydite.

The same effects were noted when Cosmos plants were grown suspended in jars with nutrient solution. The stems were kept in position with a cotton plug, in which no roots developed. Aeration of the culture solution did not give any improvement of the poor growth, and could not offset the chlorosis which developed both in aerated and unaerated solutions. Within one week after lowering the nutrient solution to 6 cm. below the top of the jars the growth became normal again, but only in those plants which had a sufficient number of young roots developed in the air above the nutrient solution. Also in this case the stem growth rate was determined by the extent of root development outside the nutrient solution.

Discussion

It seems that the previous experiments are sufficient to draw the following conclusions: A tomato plant with all of its roots submerged in a complete nutrient solution will grow slowly and may develop a chlorosis which cannot be cured by increased doses of iron and minor elements, even when sprayed on the leaves. Aeration of the solution improves the development of the roots, but aeration itself cannot cure the condition of stunted growth and chlorosis. This poor growth is *not* a result of insufficient water or salt uptake; at no time was wilting of plants observed. From table V it follows that the sugar and the osmotic concentration of plants growing slow and fast was the same, so that apparently their salt concentration was also the same. This is brought out more clearly by the experiments with divided root systems. The plants do not become normal and healthy before roots develop outside the solution. But then half the root system in solution is sufficient to take up all the water and salt required for good growth, whereas, beforehand double this amount of roots seemed insufficient. The effect is so marked and appears so soon after transfer of the roots that an indirect effect of the roots in air on those in solution seems highly improbable. Effects due to better aeration of the root system in water through oxygen supplied by the roots in air are excluded since (1), aeration of the solution decreases rather than increases top growth; (2), the two portions of the root system are separated by 10 cm. of split hypocotyl, and these halved hypocotyls do not show development of aerenchyma. Therefore, we must conclude that the portion of the root system in solution was perfectly capable of taking up all necessary salts and water, but that the top was unable to utilize them without the help of roots in air. The experiments described above have shown that although the roots in the solid medium are able to take up water, the bulk of the water uptake occurs by the roots in nutrient solution. Since the roots outside the nutrient solution have practically no

salt uptake, and cause increased growth even though they cannot take up organic materials (when grown in haydite, silica gravel, or sand), their effect can only be due to internal secretion of a factor required for satisfactory top growth. This same conclusion has been reached in the case of seedlings (**14, 15**), and this factor was named caulocaline. It is possible that caulocaline is a complex of factors; for further discussion the reader is referred to WENT and BONNER (**17**), where evidence of the chemical nature of caulocaline is produced. If we piece all present knowledge together, we can conclude: Roots supply a factor (or factors) to the growing region of the shoot, indispensable for stem growth, and for convenience sake named caulocaline. In many plants this caulocaline is formed only in roots surrounded by moist air. It travels upward in the stem, apparently under the influence of auxin (**16**), through the living elements of the vascular bundles (**5**) and has not been extracted in large quantities as yet.

Let us ask whether this knowledge about the formation of caulocaline is useful in explaining other well-known phenomena. In the first place, we have to bear in mind that the individual differences of various plants are enormous as far as the air requirement around their roots is concerned. Many plants such as rice, *Ranunculus sceleratus*, and *Cyperus alternifolius* (**2**) can grow with all of their roots submerged; but others, like tomato, must have part of their root system in contact with air to produce maximal growth. GERICKE (**4**) specifically mentions that in roses "the root crown should never be immersed in the liquid solution." This excessive aeration of the root crown is *not* required because otherwise no salts and water can be taken up; the oxygen requirement of the roots for salt absorption is much less than that for increasing the growth rate of the stems and for preventing the type of chlorosis described above.

Many plants require a very light and loose top soil. If the upper soil layers are allowed to pack closely together, growth in these plants is stunted. Although in most plants the roots, especially those taking up water and salts, are located deep down in the closely packed soil, still a superficial cultivation of the soil around such a plant will decrease growth if the superficial roots have been injured. This must be due to the necessity of the root crown for growth, because this cultivation does not appreciably change the conditions around the absorbing roots which are in the main below the cultivated portion of the soil.

The knee-roots, or pneumatophores, of the mangrove vegetation have long been considered to serve for air intake and gas exchange in general between the roots down in the mud and the air (**7**). Although it was physically impossible to get any considerable amount of gases exchanged over such a long distance (only through diffusion in the wide intercellular spaces of the pneumatophores) their respiratory function was generally accepted until TROLL and DRAGENDORFF (**8**) proved by direct measurements that no gas exchange of importance occurred through the pneumatophores of Sonneratia. It seems logical, therefore, to assume that these pneumatophores are necessary

for the caulocaline production required for stem growth. This view is strengthened by the observation of KARSTEN (**7**) that in the mangrove vegetation the trees with the largest pneumatophores have the largest growth rates.

In considering the bearing of caulocaline production on the growing of plants in general, water cultures have to be discussed. For 80 years plants have been grown with their roots immersed in nutrient solutions, and in the presence of all necessary inorganic elements satisfactory, although often slow growth was obtained. Proper aeration of the nutrient solution greatly increased growth in many plants. HOAGLAND and ARNON (**6**) have shown that with vigorous aeration tomato plants can grow as rapidly in water cultures as in good soil. GERICKE (**4**) suggested a modification of the water culture method consisting of supporting the plants above the nutrient solution in a seed bed containing some porous material, organic or inorganic. GERICKE's other improvements over the regular water-culture technique, such as the use of commercial salts and tap water, are adaptations of minor significance. A scientific explanation, however, is lacking for the advantages of hydroponics over the traditional water culture. Probably this is the reason why the importance of the seed bed is not generally recognized. In a pamphlet, BALL (**1**) states that hydroponics was no success in the East and Middle West, giving as the probable reason: "the Gericke plan furnished everything the soil did (see above) except air at the roots."

The experience gained with the foregoing experiments does not support the generally held views as expressed by HOAGLAND and ARNON (**6**): "While the use of a porous bed instead of a perforated cover facilitates aeration of roots, the bed can be dispensed with if provision is made to bubble air through the nutrient solution." This may be true for certain plants, but not for all. In a commercial greenhouse near Pasadena using the water culture method no other provision is made for aeration of the nutrient solution beyond pumping it slowly through the tanks. Since growth of tomatoes in this greenhouse is excellent, it indicates that aeration of the culture solution is not so essential if a proper root system has developed in the seed bed or between seed bed and culture solution.

We thus reach the conclusion that the essential improvement of hydroponics over the old water culture method is to divide the functions of the root system: one root system takes up water and salts; the other, in the seed bed or between solution and seed bed, supplies caulocaline. And this is essentially the same division of labor as we encounter in most trees and perennials. They also develop a long fibrous feeding root system, which penetrates deep into the soil, and in addition they have a root crown which is well aerated, but due to its position, cannot well serve for water and salt uptake.

Summary

It has been shown that if all the roots of a tomato (or Cosmos) plant be

submerged in a nutrient solution of pH 6 or higher, aeration cannot prevent chlorosis and especially a drop in the growth rate of the stems, although root growth is satisfactory. As soon as a portion of the root system develops in moist air, however, growth of the stem becomes maximal. All experiments point toward the conclusion that the part of the root system which develops in moist air supplies one or more factors (tentatively named caulocaline) required for stem growth and prevention of chlorosis. Thus, in intact plants, the aeration of roots seems to be of relatively greater importance for their caulocaline production than for salt uptake.

WILLIAM G. KERCKHOFF LABORATORIES
CALIFORNIA INSTITUTE OF TECHNOLOGY
PASADENA, CALIFORNIA

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USE OF TENSIOMETERS IN MEASURING AVAILABILITY OF WATER TO PLANTS

F. J. Veihmeyer, N. E. Edlefsen, and A. H. Hendrickson

(WITH ELEVEN FIGURES)

Efforts by several investigators to measure availability of water to plants by vapor pressure (**2, 12**) and other methods (**10, 14**) have indicated that the potential of water at the permanent wilting percentage is approximately 16×10^6 ergs per gram, whereas others (**1, 11**) have obtained results which indicate somewhat lower values. A great many experiments (**3, 4, 5, 6, 13**) have demonstrated that soil moisture seems to be equally available to plants at all times, as measured by the response of plants, as the moisture content is decreased from a high value to about the permanent wilting percentage. This conclusion seems to be general for all plants which have been studied. Evidently plants do not respond markedly enough to show any change in availability of water until the soil moisture is reduced to about the permanent wilting percentage. On the other hand, physical measurements show that the energy required to remove water from the soil, changes materially as the soil-moisture content decreases. It was surprising therefore, when Rogers (**9**) found, while working with strawberry plants in pots, that the plants wilted severely at a tension of 60 cm. of mercury (potential of 0.8×10^6 ergs per gram). Furthermore, his illustration shows plants wilted slightly at 47 centimeters (0.6×10^6 ergs per gram). The maximum potential measurable by tensiometers is less than 1×10^6 ergs per gram. The work reported herein was undertaken largely in an effort to explain this apparent discrepancy.

Experiments with sunflowers and strawberries

Since much of the work at Davis had been done with sunflowers, the first trial was made with these plants. The plants were grown in a metal container holding approximately 15 kilograms of soil. A tensiometer connected to a mercury manometer was inserted near the center of the container. The plants were allowed to dry the soil to the permanent wilting percentage several times before the experiment was started.

Figure 1 shows the tension as a function of average moisture content of the soil. The permanent wilting percentage and the moisture equivalent are given. The potential at the maximum reading of the gauge (56 centimeters of mercury) is approximately 0.8×10^6 ergs per gram. Figure 2 shows the appearance of the sunflowers when the tension was near the maximum value. Clearly the sunflowers were fully turgid; furthermore, they remained so until the permanent wilting percentage was reached. The gauge did not operate at the lower moisture contents. This disagrees with the results published by Rogers (**9**). He worked with strawberry plants, however, so

it was decided to see if by some chance these plants behaved differently than sunflowers. Strawberries were accordingly planted in double-walled burned clay jars (7), the inside wall of which was porous. The arrangement is shown in figure 4. Samples of soil to determine the moisture content in the jar were taken at various times with a cork borer, the holes being refilled with soil at approximately the same moisture content. The top half of the sample was kept separate from the bottom half and the average moisture content of the top half as well as the bottom half of the jar are plotted as a function of time in figure 3. The moisture equivalent and the permanent wilting percentage of the soil are shown. The plant continued to maintain its turgidity until the permanent wilting percentage was reached. Figure 4, A, was taken immediately following an irrigation so that all the

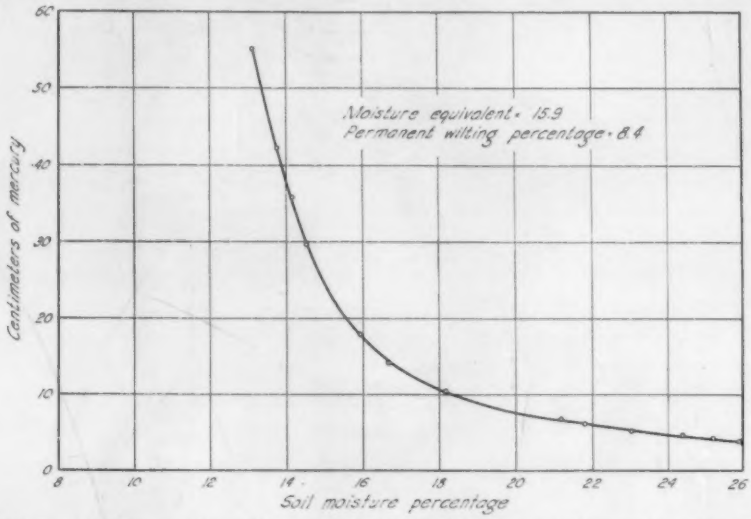

FIG. 1. Tension of the water expressed in centimeters of mercury as a function of moisture content for Yolo silt loam on which the sunflowers (292) were growing.

soil was wet and the gauge reading showed zero at the start of the experiment. Figure 4, B, shows the gauge reading 25 inches of mercury (potential of 0.8×10^6 ergs per gram) and the condition of the plant at this tension. There is no indication of wilting at this maximum tension read on the gauge.

As shown in figure 3, there was some difference in moisture content between the top half of the jar and the bottom half even though lead foil was used to cover the surface of the soil to retard evaporation. While there were variations in moisture contents in different portions of the soil, that at the center of the pot was not consistently higher or lower than at the edge. To test the distribution of tension within the jar a small porous cup was placed approximately in the center of the soil mass and connected to a gauge as shown in figure 5. The pot was then irrigated and the plants were allowed to reduce the moisture until the tension of 25 inches of mercury was

reached. No corrections have been made for the difference in elevation of the gauges above the center of the porous vessel containing the water, but it is clear that the readings on the two gauges are approximately the same; that is, the tension at the outside wall is practically the same as in the center. This was to be expected from our measurements on the horizontal distribution of moisture within the pot as reported above. Our results indicate that strawberries are no different from other plants studied in their ability to use soil moisture from soil permeated by their roots. Our results, never-

FIG. 2. Sunflowers (292) near the maximum obtainable tension of the water in the tensiometer. They appear to be the same as the sunflowers (209) growing on soil above field capacity.

theless, differ markedly from those of ROGERS (9). A possible explanation is that since ROGERS grew his plants in single-walled porous flower pots, evaporation from the wall had dried the outer part of the soil mass to a greater extent than the center of the pot where the porous cup used to measure the tension was located.

A further study was made of the use of the tensiometer with strawberries under field conditions. The porous cup was connected to a tension gauge and placed with its center about 18 inches below the surface of the soil in a

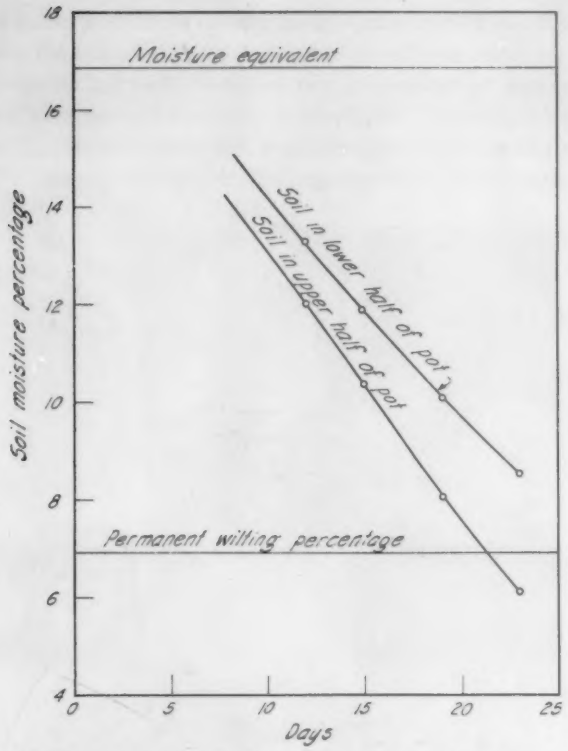

FIG. 3. Soil-moisture in the top half and bottom half of the double-walled pot shown in figure 4 containing Madera silt loam.

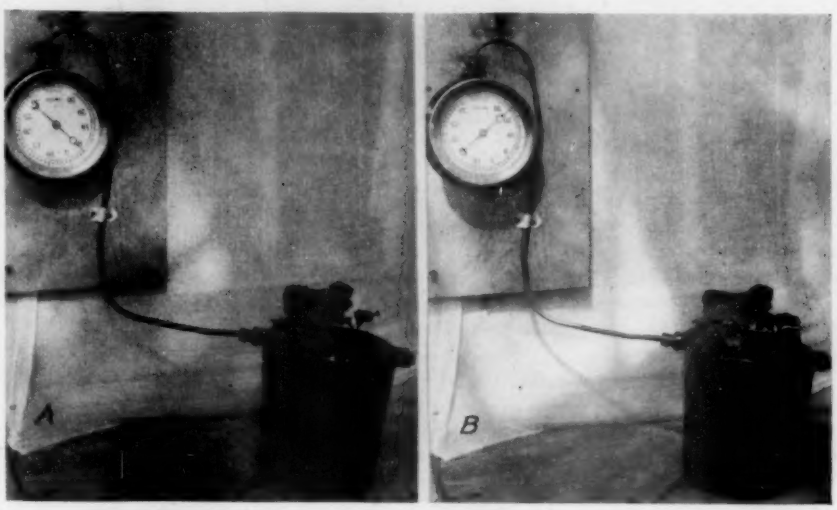

FIG. 4. Strawberry plant in a double-walled porous pot: (A) at the beginning of the experiment when the soil was wet; (B) the same plant when the tension of the water in the tensiometer had been increased to 25 inches of mercury. At this tension, there was no evidence of wilting.

strawberry bed. Moisture samples were taken at 6-inch intervals to a depth of 2 feet. It is commonly thought that strawberries are extremely shallow-rooted plants and, therefore, it was assumed that 2 feet would be ample depth at which to sample. Figure 6 shows four curves, one for each 6-inch depth, giving the moisture content as a function of time. The permanent wilting percentage and moisture equivalent are also given.

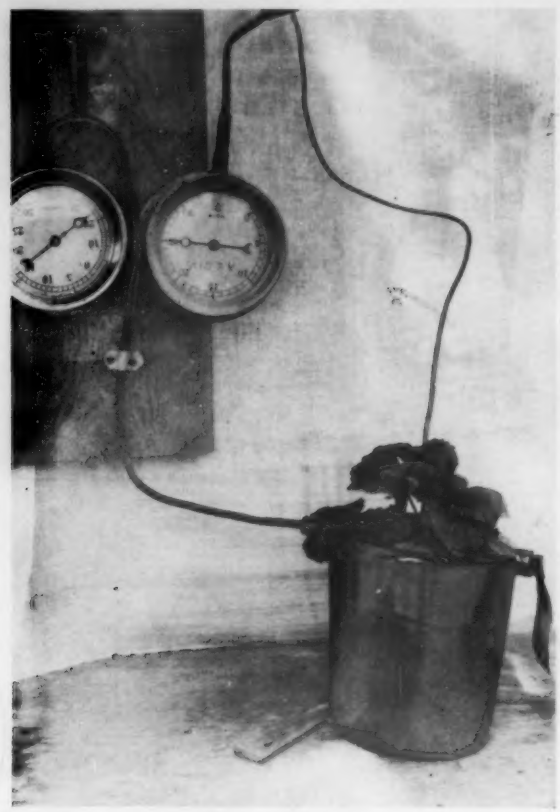

FIG. 5. The readings of two gauges, one of which measures the tension of the water between the two walls of the porous pot; the other is attached to a porous cup placed in the center of the soil mass. This arrangement was employed to determine whether there was an appreciable difference in the tension between the center and the outside of the soil mass. The readings were so close that no differences could be observed.

In figure 7 are shown photographs of the strawberries at the three different times indicated in figure 6 by the three vertical lines. The picture marked A was taken immediately following an irrigation. The photograph marked B shows the strawberries when they had dried the soil sufficiently so that the gauge, which had been installed following irrigation, had reached approximately 25 inches of mercury (potential of 0.8×10^6 ergs per gram), no correction being made for the water column from the center of the cup to the center of the gauge. As the plants continued to use water, air grad-

ually leaked in so that the reading dropped to zero by the time the picture marked C was taken; at this time some of the plants were just beginning to show some wilting, although the maximum reading on the tensiometer had been reached 27 days previously. In fact, the plants continued to remain turgid after all the readily available water had been used out of the top 2 feet of soil. This was surprising in view of the fact that it is usually assumed that strawberries are shallow-rooted plants.

In an attempt to find out what the approximate root zone of the strawberry plant was, the bed was irrigated again and samples were taken at

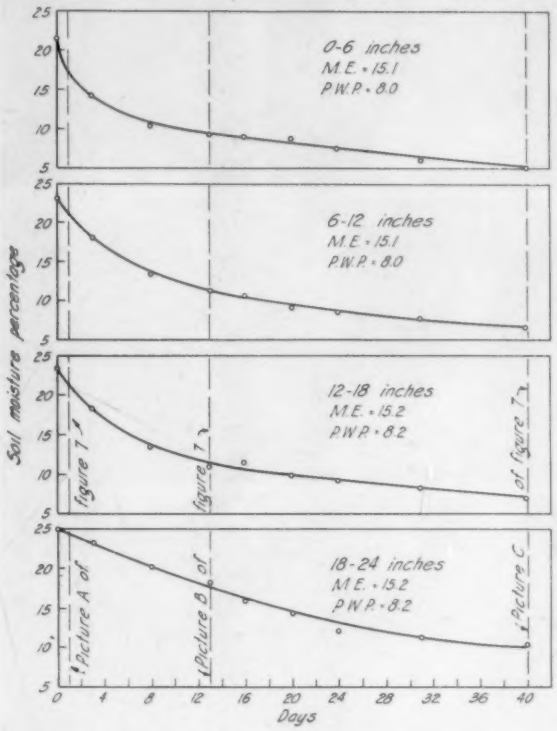

FIG. 6. Moisture content as a function of time for each 6-inch layer to a depth of 2 feet in the strawberry field shown in figure 7 on Yolo fine sandy loam. The three vertical lines show the times at which the photographs, figure 7, were taken.

1-foot intervals to a depth of 4 feet. The curves in figure 8 show the moisture content as a function of time for each foot section down to 4 feet. Apparently, some water was extracted below 3 feet. The conditions of the plants at various times are shown in figure 9. The picture marked A was taken immediately following an irrigation. The picture marked B was taken when practically all the available water was used from the top 2-foot section, whereas the picture marked C was taken at the end of the experiment. As can be seen, the plants show little need of water even at the end of the experiment. An appreciable amount of water was used from the 4th foot.

Replicability of tensiometer readings

Further studies on the use of tensiometers under field conditions were made on a Sudan grass plot. The tensiometers used here were constructed under the direction of Dr. L. A. RICHARDS (8), and, through his kindness, loaned to us for the experiment. Tensiometers were placed in the center of

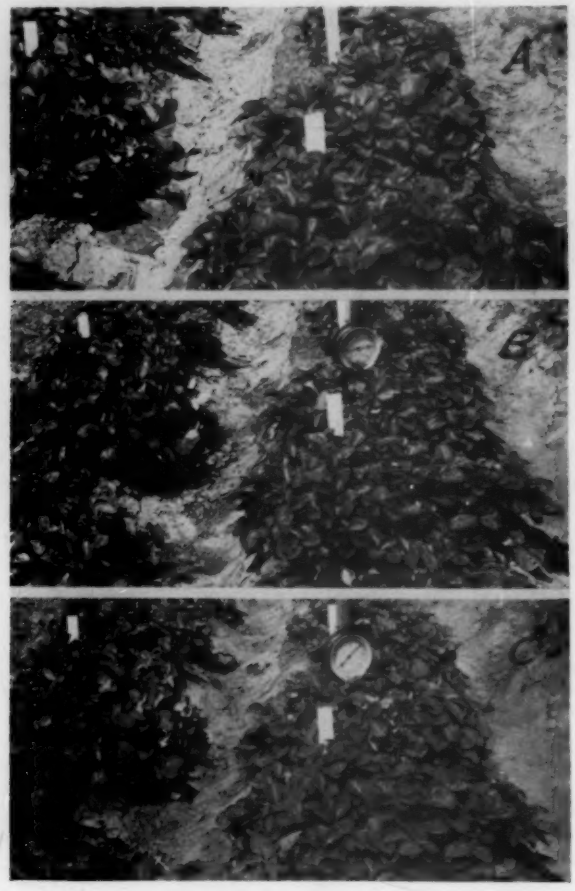

FIG. 7. (A) Strawberry plants immediately following an irrigation; (B) when the tension of the water in the porous cup attached to the gauge had reached approximately 25 inches of mercury; (C) when practically all of the readily available water had been used from the top 2 feet of soil. The tensiometer reading was approximately at its maximum value at the time B was taken. Air had leaked in and the gauge had dropped to zero by the time C was taken.

the first-, second-, third-, and fifth-foot depths. Variations of moisture content and tension with time for each depth are shown in figure 10, the tension being plotted to a logarithmic scale. The moisture equivalent and permanent wilting percentage are given for each depth.

At no time during the season did the Sudan grass show any indication

of need for water. When irrigation was applied, it was done not because the grass showed need for water, but in an effort to start the field from a wet condition again so that a check on the replicability of the tensiometer could be made. It should be mentioned that enough water was applied to this field to wet the soil to the water table 10 feet below the ground surface, and hence the soil below the top foot remained well above the moisture equivalent for a long period after the second irrigation. To test the replicability of the tensiometer readings for different "cycles,"[1] the curves of figure 11 were derived from data taken from the curves of figure 10. They show the tension as a function of moisture for each of the foot sections studied.

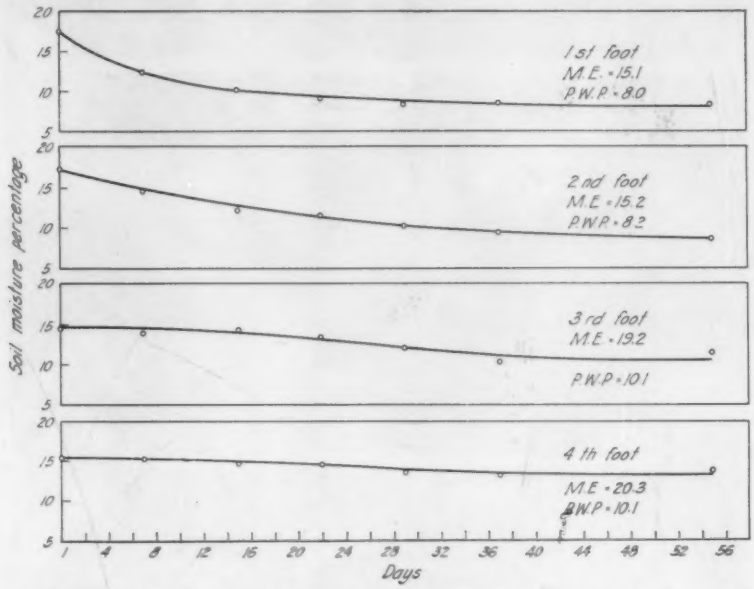

FIG. 8. The soil-moisture content of the top 4 feet of soil in the strawberry field shown in figure 9. This is the same field shown in figure 7.

The moisture equivalents for each foot are shown on the graph. It is, of course, impossible to use the data for such a curve at tensions higher than where the tensiometer operates, and hence the moisture content does not decrease to as low a value as it does in the curves of figure 10. The tension increases very rapidly as the moisture content decreases in the neighborhood of the moisture equivalent. This makes it extremely difficult to fix with any degree of exactness the value of the tension at the moisture equivalent. However, as can be seen from the curves of figure 11, a tension of 300 centimeters of water (0.29×10^6 ergs per gram) is approximately the value when these soils are at the moisture equivalent. This is in substantial agreement with RICHARDS (7). It is considerably lower than most

[1] By a cycle we mean the sequence of moisture changes which the soil passes through from one irrigation to another.

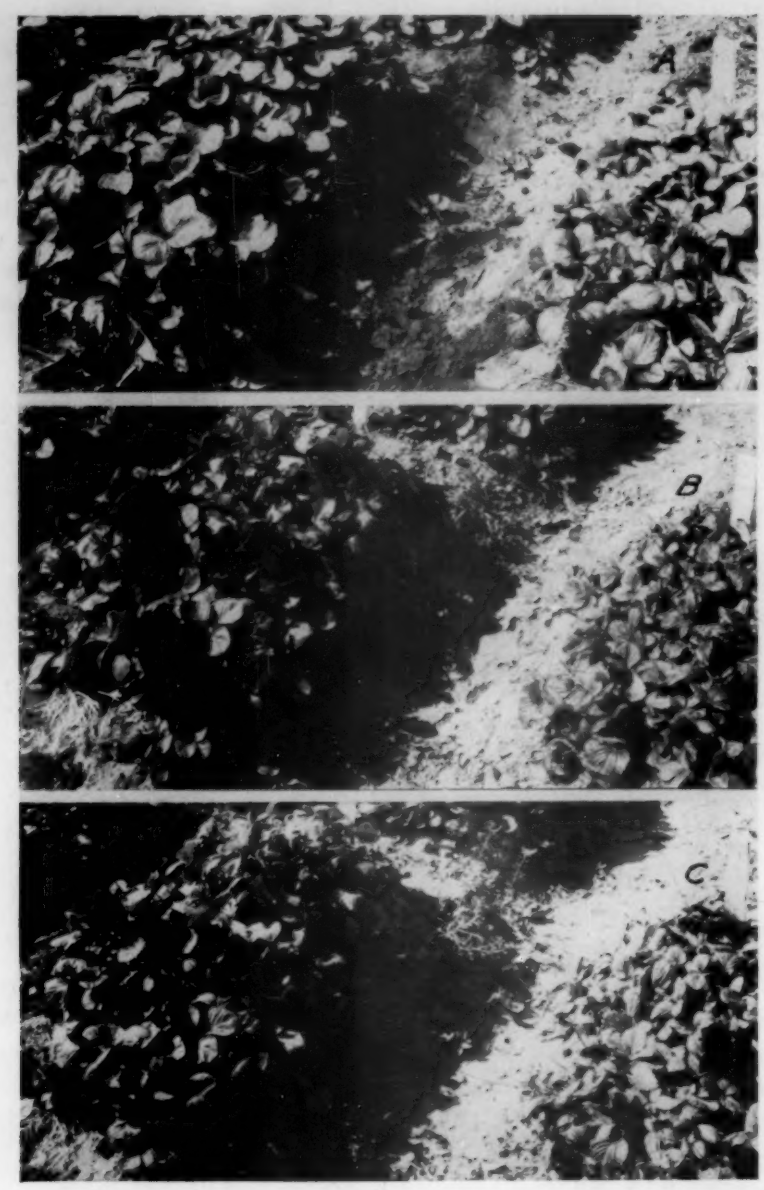

FIG. 9. (A) Strawberry plants immediately following irrigation; (B) 30 days after irrigation, and (C) at the end of the experiment 55 days after irrigation. The plants in B appear to be as turgid as in A.

of the values estimated by SCHOFIELD (**10**) who concluded from available data, that the pF was between 2.5 and 3.0 (0.31×10^6 to 0.98×10^6 ergs per gram) at the moisture equivalent.

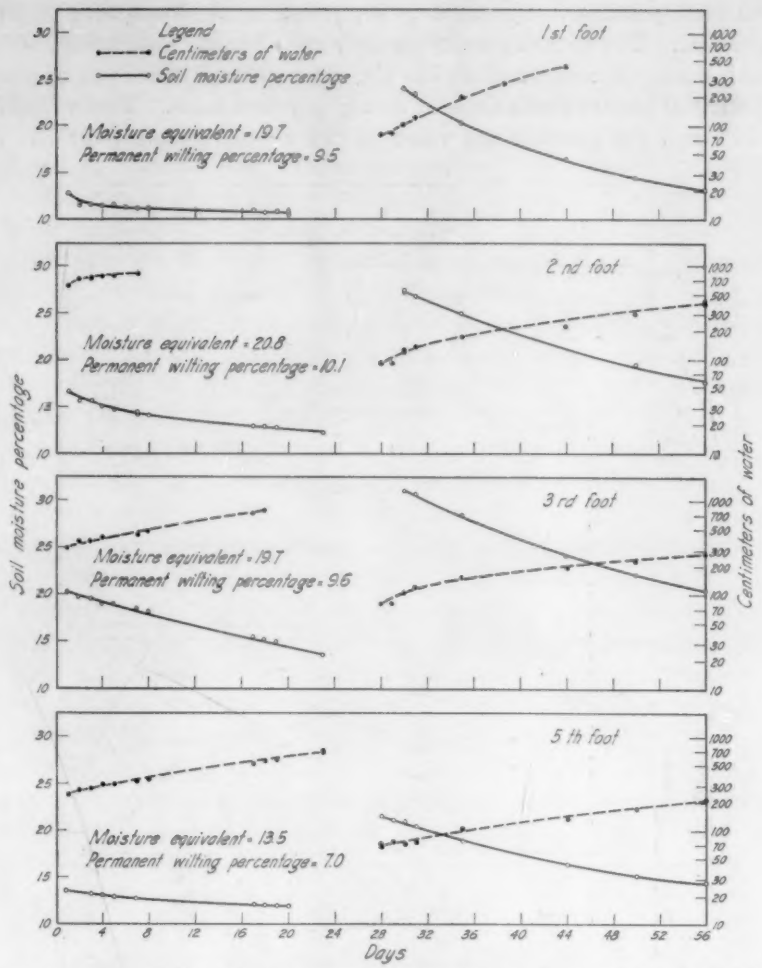

FIG. 10. The tension of the water in the tensiometers expressed in centimeters of water and the moisture content in a Sudan grass plot as a function of time for the first, second, third and fifth-foot depths.

A confusion between numerical and dimensional equality in certain soil-moisture measurements

In this connection, it is worth while calling attention to an error which is frequently made. Since the moisture equivalent is approximately equal to the field capacity (which is usually considered to be the moisture held in the soil against the pull of gravity) of most fine-textured soils but not always sands, and since in the c.g.s. system the pull of gravity on unit mass is *numerically* equal to the pressure of 1 atmosphere, it has been reasoned by some that the potential at the moisture equivalent is equal to a tension of 1 atmosphere, or a pF of 3, or 1.0×10^6 ergs per gram. This is faulty reasoning. The fact that in the c.g.s. system the pull of gravity on unit mass is

approximately numerically equal to a pressure of 1 atmosphere is purely coincidental. The pull of gravity on unit mass has the dimensions of force per unit mass, whereas pressure has the dimensions of force per unit area; and potential has the dimensions of energy per unit mass. That pF of 3, or 1.0×10^6 ergs per gram is the value at the moisture equivalent has been

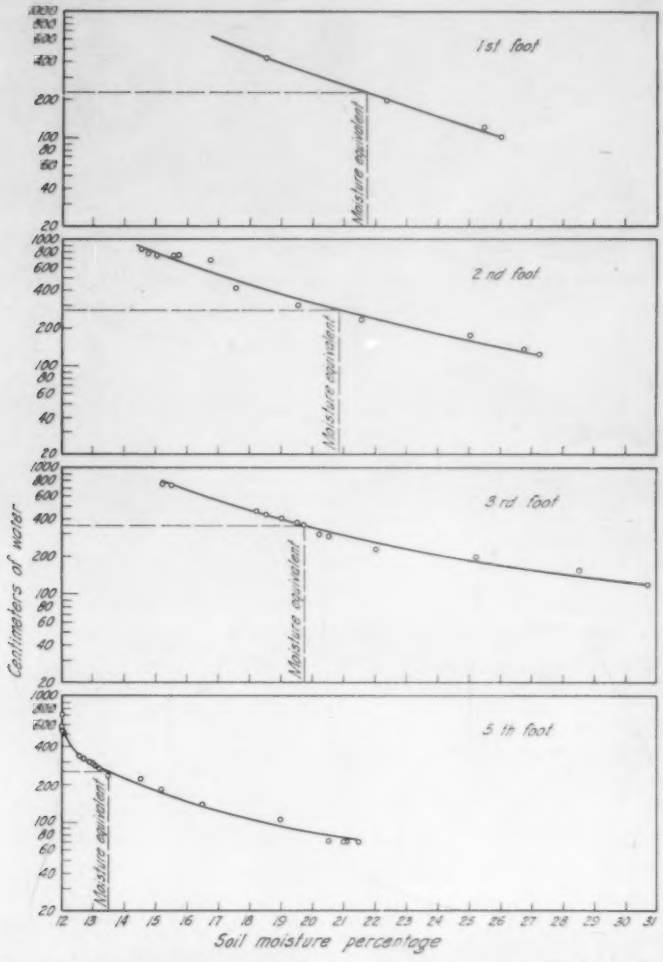

FIG. 11. The tension of the water in the tensiometers expressed as centimeters of water as a function of moisture content for the first, second, third, and fifth-foot depths in the Sudan grass plot, the moisture content of which is shown in figure 10. The tension at the moisture equivalent is indicated.

given added credence because it coincided with the upper range of values for pF at the moisture equivalent as estimated by SCHOFIELD. There is also another reason why a pF of 3 has been associated with the moisture equivalent. By assuming that a potential of zero exists at the outer surface of a soil sample one centimeter thick in a centrifuge being subjected to a centrifugal field of 1000 times gravity, it follows that the potential at the

inside surface is 1.0×10^6 ergs per gram or a pF of 3. The average pF for the sample is much less than this.

Summary

Pot and field experiments using sunflowers, strawberries, and Sudan grass indicate that tensiometers are capable of indicating the tensions for moisture contents in the upper one-third to one-half of the range between the moisture equivalent and the permanent wilting percentage.

Strawberry plants in pots remained turgid when the roots were in soil at moisture contents much lower than those which would produce a maximum tension of about 700 centimeters of water. Similarly, the moisture content in the top 3 feet of soil in the strawberry field reached a percentage lower than that which would show a tension of about 700 centimeters of water and yet the plants remained turgid.

All of the soil in the pots and in the top 3 feet of the strawberry field plots was reduced to the permanent wilting percentage before there was any evidence of wilting. At this moisture content there would be an equivalent tension of about 16,000 centimeters of water as estimated by available data on the potential of the water at the permanent wilting percentage.

Tensiometers in the Sudan grass plots showed close replicability for different "cycles." Tensions obtained were about the same even though the soils tested varied in texture as measured by the moisture equivalent.

For the soils tested, the potential at the moisture equivalent, while difficult to evaluate exactly, is in the neighborhood of 0.3×10^6 ergs per gram.

Attention is called to an error in reasoning occurring in the literature in regard to evaluating the pF and the potential of soil moisture at the moisture equivalent.

THE UNIVERSITY OF CALIFORNIA
DAVIS, CALIFORNIA

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MEASUREMENT OF SMALL CONCENTRATIONS OF ETHYLENE AND AUTOMOBILE EXHAUST GASES AND THEIR RELATION TO LEMON STORAGE

P. W. ROHRBAUGH

(WITH FIVE FIGURES)

Introduction

Ethylene, even in very small concentrations, is known to hasten the coloring and ripening of fruits and vegetables in storage and to greatly increase their rate of respiration.

Further study of this problem has been made for the purpose of obtaining a satisfactory test for the detection of small concentrations of ethylene in connection with commercial storage of lemons. It was desirable to know what concentrations of ethylene accumulate in lemon storage rooms from decaying fruit; also what might be the importance of motor exhaust gases from loading and unloading trucks or from the cars and trucks of nearby highways.

Probably the earliest written record of the effects of motor gases on fruit is given in a paper by SIEVERS and TRUE (**17**). They reported that lemons were being colored quickly in the packing house by piping the exhaust gases from a gasoline engine into the coloring rooms. They also stated that the exhaust gases produced a very satisfactory fruit color in the same time as was required with kerosene stove fumes. Apparently the fruit was colored as quickly as it now is with ethylene.

WRIGHT (**18**) describes methods of using motor exhaust gases for coloring oranges. This author states that the gas in a well-regulated coloring room should be just strong enough that a person experiences only a slightly disagreeable odor or burning sensation of the eyes. His observation was that the first sensation experienced on entering the room is the most reliable. He recommends that the gas be run into the room during only about 10 hours a day.

BARGER and HAWKINS (**1**) have described the satisfactory use of gasoline engine exhaust fumes for coloring grapefruit in Florida. DENNY (**5**) after analysis of kerosene stove fumes concluded that the ethylene fraction was mainly responsible for the coloring of fruit and stated that ethylene when used in concentrations as low as 1 p.p.m. brings about this coloring. He also pointed out that a wide range of ethylene concentrations from 1 part in 2 million to 1 part in 5000 may be used to color fruit.

MACK (**14**), working on the blanching of celery with ethylene, found that 20 p.p.m. to 40 p.p.m. gave the optimum concentration for blanching and concentrations more than 40 p.p.m. gave less blanching.

It has been shown that a number of different fruits give off ethylene gas during ripening. While ELMER (**6**) appears to be the first to point out

that apples give off some form of physiologically effective gas, it remained for GANE (7) to prove that the gas was ethylene. Other fruits have also been found to give off ethylene. NIEDERL *et al.* (16) found that bananas give off small amounts of ethylene while ripening. HANSEN and HARTMAN (10) in 1935 gave considerable evidence that ethylene was given off by pears. HANSEN (9), again working on pears and measuring the ethylene by chemical means, studied the relation of ethylene production to respiration. He found that the amount of ethylene given off by the ripening of pears was influenced greatly by such factors as temperature, anaerobic conditions, and varietal differences. BIALE (2) and MILLER *et al.* (15) have shown that emanations, believed to be ethylene, are given off by citrus fruit decaying from the common green mold, *Penicillium digitatum* Sacc. MILLER *et al.* (15) also found that sound citrus fruit gave slight but positive tests for ethylene.

Without having some delicate simple test, it is impossible to know when storage rooms are properly ventilated or whether the air being taken into the storage rooms is free from ethylene. It has been our purpose, therefore, to develop a method which could be used to test automobile exhaust gases for ethylene and to detect small amounts of this gas in fruit storage rooms.

Method

The epinasty test using pea plants, with some modification of the method as described by KNIGHT *et al.* (12) and KNIGHT and CROCKER (11), seemed to offer the best possibility as a method of quantitatively measuring very small ethylene concentrations.

Results

MEASUREMENT OF THE ETHYLENE EQUIVALENT OF MOTOR EXHAUST FUMES

Alaska peas were planted in cans 2½ inches high and 4 inches in diameter, the cans being filled with one part sand, two parts water and three parts peat moss. The peas were grown at 75° F. until the plants were one or two inches high. This usually required four days.

One can of peas was placed in each of eight removable lid steel drums of 55-gallon capacity. After the peas were placed, the lids were clamped tightly and different measured quantities of motor exhaust fumes were placed in five of the drums; measured quantities of ethylene were placed in two drums, and one was kept as a control. After four days, peas grown in the exhaust fumes were compared with the others grown in the drums with known quantities of ethylene and with the control to which no gas had been added.

The automobile exhaust gases to be tested were first introduced into an ordinary steel drum which served only as a reservoir. This was done by means of a 1¾-in. hose one end of which was placed on the exhaust of a Ford V8 automobile and the other end into the side opening of the drum. The top of this drum contained a four-inch opening from which the gases

could escape. The automobile motor was started and the gas fumes allowed to run into the drum for about 15 minutes to displace the air. The rate of flow of the gas from the motor was measured by having an anemometer on the opening in the top of the drum and the quantity of exhaust gases coming from the motor in a given time was recorded. A single barrel tire pump had been made into a syringe by removing the valves, placing a double leather on the plunger and calibrating it. The pump was used to remove given quantities of the motor gas from this drum and place it in the drums containing peas.

A known amount of ethylene was introduced into two of the drums as follows. The ethylene was first introduced into a 5-gallon carboy to give a

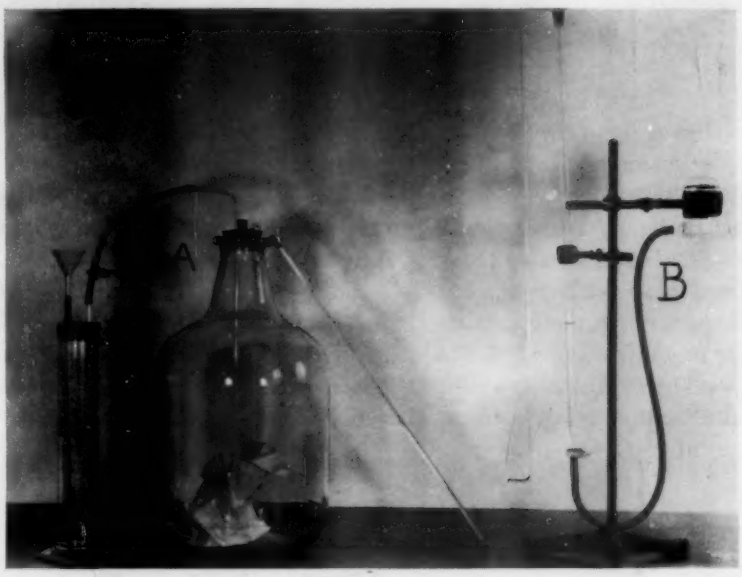

FIG. 1. Equipment used for measuring out small quantities of ethylene into epinasty test drums.

concentration of 1 part ethylene to 2000 parts air mixture. This was done by means of water displacement from a burette having a 0.5-mm. capillary tube connected at the top and a leveling bottle at the bottom. The leveling bottle was raised to fill the burette and capillary with water. Then the capillary was attached to the ethylene container and the water in the capillary and most of that in the burette were displaced with ethylene. The capillary was then connected to the carboy and the desired amount of ethylene introduced by raising the leveling bottle (see fig. 1B). The carboy also contained a piece of crumpled paper which helped to mix the gas contents when the carboy was shaken. The desired amount of this diluted gas was then introduced into the barrels containing the pea plants by connecting a closed graduated cylinder with a funnel and a capillary tube. The capillary tube was inserted into the bung hole of the drum. By adding a given

quantity of water to the graduated cylinder air was forced into the carboy, displacing an equal amount of the gas mixture from the carboy into the drum with the peas. Direct water displacement was avoided since ethylene is appreciably water-soluble (see fig. 1A).

The ethylene and the concentrations obtained in the drums are given in table I.

TABLE I

Drum number	Gas added	Amount of gas added	Concentration in drum
		ml.	*p.p.m.*
1	None	.....	.....
2	Ethylene	0.05	0.25
3	Ethylene	0.1	0.5
4	Motor exhaust	25.0	120.0
5	Motor exhaust	80.0	400.0
6	Motor exhaust	160.0	800.0
7	Motor exhaust	400.0	2000.0
8	Motor exhaust	800.0	4000.0

Figure 2 shows a set of peas after four days in these drums. There was no bending due to epinasty in the control drum although the plants were tall and etiolated so that when removed for photographing several of them bent over from their own weight.

The peas in drum no. 2 with 0.25 p.p.m. of ethylene bent over and grew 2″ to 2½ in. horizontally. Those in no. 3 with 0.5 p.p.m. ethylene bent over and grew 1½ to 2 in. horizontally. Those in no. 4 with 120 p.p.m. of motor gas showed almost as much epinasty as those in 0.25 p.p.m. of ethylene. The

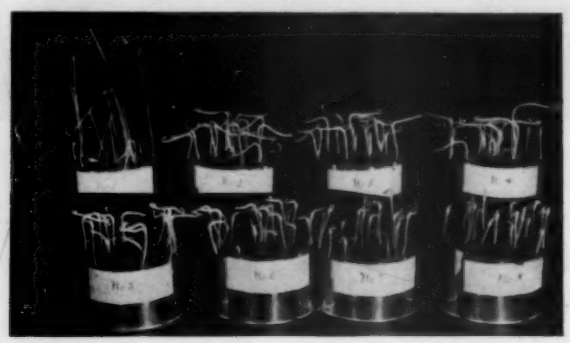

Fig. 2. Epinasty produced by ethylene and motor exhaust gases on Alaska peas.

No. 1. Control (several peas bent over by their own weight after removing from test drum).
No. 2. 0.25 p.p.m. ethylene
No. 3. 0.5 p.p.m. ethylene
No. 4. 118 p.p.m. motor exhaust
No. 5. 400 p.p.m. motor exhaust
No. 6. 800 p.p.m. motor exhaust
No. 7. 2000 p.p.m. motor exhaust
No. 8. 4000 p.p.m. motor exhaust

peas in drums nos. 5, 6, 7, and 8 showed progressively less plant growth. No. 8 showed almost no growth at all. The last four showed considerable swelling or enlargement of the stems.

From these results it appears that no. 4, with 120 p.p.m. of motor exhaust gas, was affected slightly less than no. 2 with 0.25 p.p.m. of ethylene. No. 5, with 400 p.p.m. of exhaust gas, was affected much more than no. 3 with 0.5 p.p.m. of ethylene. It would appear that drum no. 5 has about twice as high a concentration of ethylene as no. 3.

If we consider that no. 4 with 25 ml. of motor exhaust gas is the equivalent of no. 2 with 0.05 ml. of ethylene and that no. 5 has twice the ethylene equivalent of no. 3 we can calculate the ethylene equivalent of the motor fumes as follows:

0.05 ml. ethylene = 25 ml. motor exhaust gas.
1 ml. ethylene = 500 ml. motor exhaust gas.
2000 ml. ethylene = 1,000,000 ml. motor exhaust gas or 2000 p.p.m.

The peas in no. 4 did not bend as sharply, and they grew a little longer than those in no. 2, indicating that the ethylene equivalent in no. 4 was not quite as great as that of no. 2. The ethylene equivalent of the motor gas in barrel no. 4 was probably more nearly equal to 0.04 ml. of ethylene; and assuming that the ethylene quivalent of the exhaust gas in the air in barrel no. 4 was equal to 0.04 ml. ethylene, then the exhaust gas has an ethylene equivalent of 1600 p.p.m. instead of 2000 p.p.m. The ethylene equivalent of the motor gas is then in the range of 1600 to 2000 p.p.m.

It was found that the car gave off 529 liters of exhaust gas per minute when idling. If this gas has an ethylene equivalent of 1800 p.p.m., then $\frac{529{,}000 \text{ ml.}}{1{,}000{,}000} \times 100 = 952$ ml. per minute of ethylene equivalent are given off by the car.

Relation of epinasty to lemon coloring

Another experiment was set up in which 12 green lemons were placed in each of the 8 drums. The fruit for this test was selected as carefully as possible to get the same color, size, and type of lemons. The same amounts of ethylene and motor exhaust fumes were used as for the peas in the previous experiment. The lemons were left 10 days at 76° F. and then the drums were opened and the lemons examined for color.

The control fruit in no. 1 had colored very little.

The fruit in no. 2 was noticeably more colored than the control.

The fruit in no. 3 was not noticeably different from no. 2.

The fruit in no. 4 was slightly less colored than that in nos. 2 and 3.

The fruit in nos. 5, 6, 7, and 8 were progressively more colored, no. 8 almost fully colored.

These results indicate that the exhaust gases and ethylene are as effective in coloring lemons as they are in producing epinasty.

Another set of tests was run using different concentrations of ethylene. These data are given in table II.

TABLE II

Drum no.	Ethylene concentration	p.p.m.	Effect after 14 days
1 Control	None	....	Slight change in color
2	1 part to 40 million	0.025	No noticeable difference from control
3	1 part to 20 million	0.05	A little more color than control
4	1 part to 10 million	0.1	Slightly more color than in no. 3
5	1 part to 5 million	0.2	Quite well colored
6	1 part to 2.5 million	0.4	Almost fully colored
7	1 part to 1 million	1.0	Almost fully colored
8	1 part to 0.5 million	2.0	Almost fully colored

These data cover a range of concentrations much lower than those used previously but the fruit were left in for a longer time. There was no noticeable effect in 14 days by concentrations of less than 1 part in 20 million.

Figure 3 shows the effect of a similar range of concentrations of ethylene

Fig. 3. Epinasty produced by different concentrations of ethylene on Alaska pea seedlings.

on peas under the same conditions as those used on the fruit. Table II and figure 3 show that there is a very close correlation between the concentrations necessary to cause bending in peas and coloring of lemons. Any ethylene concentration which is strong enough to cause epinasty or bending of the peas will cause an appreciable increase in the rate of coloring and maturing of lemons.

Ethylene equivalent content of lemon storage rooms

More or less green mold decay always develops among lemons during the storage season. We questioned whether the ethylene given off by this decay might be an important factor in the premature coloring and maturing of the fruit; also whether the exhaust gases given off by cars and trucks and entering the storage rooms through fresh air intakes might have that effect.

Since the storage rooms are kept at a temperature of 55–60° F. it was necessary in order to run the epinasty test to devise some means of maintaining the peas in the storage room atmosphere and at the same time to keep them dark and at a temperature of about 76° F. These requirements were met by making a container consisting of a can 6″ in diameter and 11″ high with a maze effect at the bottom and in the cover so that the air could

come in around the bottom and go out at the top. This can was then equipped with a heater consisting of 2 10-watt intermediate base electric lights connected in series. Each light was covered with a collapsible metal tube to keep all light from the pea plants. This heater was placed in the bottom of the container and covered first by a piece of asbestos then by a small metal stand. On the stand was placed a can of peas previously grown in the can of peat moss and sand until 1 to 2 in. tall. The heater (shown in fig. 4) maintains the peas at very nearly an even temperature as long as the room temperature and air movement are the same. Different size lamps may be used in storages operated at other temperatures or thermostats may be employed.

This method of testing storage room air for the presence of ethylene appears to be quite satisfactory as a commercial method and is more accurate

FIG. 4. Electrically heated dark chamber for growing peas in storage rooms.

than any of the usual chemical test methods. Peas can be grown continuously in the storage room; when one set is removed others are put in their place. Figure 5 shows pictures of peas grown in different lemon rooms.

Since figure 3 shows the effects of different known concentrations of ethylene, it can be used as a guide in estimating the concentration of ethylene in storage rooms.

The ethylene concentration of storage rooms may vary a great deal, depending on the amount of fresh air being brought in; the amount of fresh air brought in depending to a large extent upon the temperature and humidity outside as well as the refrigerating capacity available.

ETHYLENE EQUIVALENT PRODUCED BY DECAYING LEMONS

The rate of production of ethylene from one decaying lemon was roughly estimated by running air at the rate of 20 liters per hour through a jar containing a lemon decaying with *Penicillium digitatum* then through another jar containing a can of the pea seedlings. The effect on the peas indicates a concentration of ethylene of about 1 part to 7 or 8 million parts of air.

This means that enough ethylene or its equivalent is added to 20 liters of air flowing through the jars per hour to equal a concentration of 1 part ethylene in about 7.5 million parts of air or $\frac{1}{7{,}500{,}000} \times 20$ liters per hour or $\frac{480}{7{,}500{,}000}$ liters per day. This is equal to 0.064 ml. of ethylene per day or

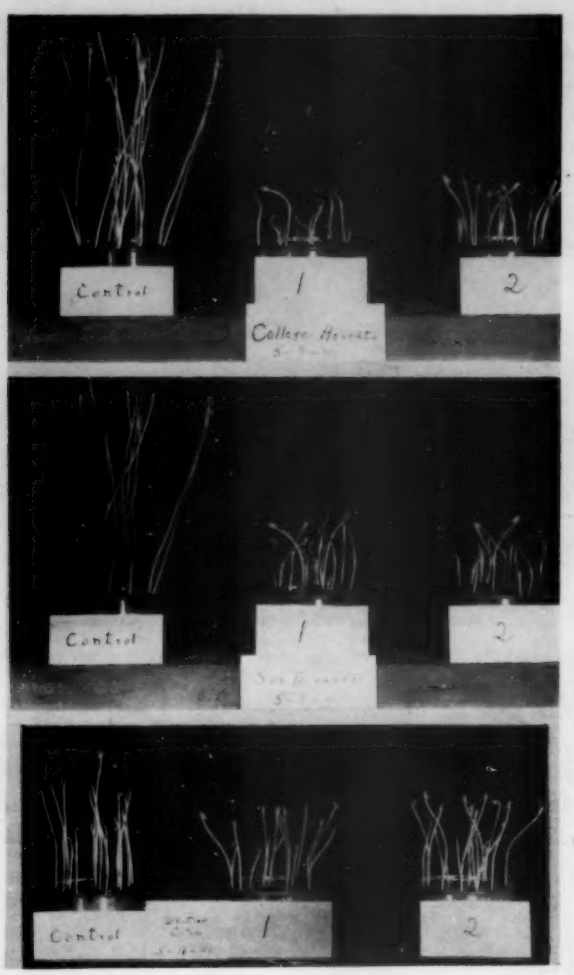

FIG. 5. Epinasty effects obtained on pea seedlings by ethylene in three different lemon storage rooms.

about 1 ml. in 15 days. While it has not been determined over how long a time this continues, it is considered probable that not much ethylene is given off after 15 days as the fruit is quite well decayed by that time.

SIGNIFICANCE OF ETHYLENE IN A STORAGE ROOM

BIALE and SHEPHERD (3) have shown that lemons give off CO_2 at the rate

of 7 to 8 mg. per kilo of fruit per hour at 58° F. or an average of 180 mg. of CO_2 per kilo of fruit per day.

The work of GREEN *et al.* (**8**) shows that the heat given off by fruit and vegetables is in direct proportion to the CO_2 given off. They also found that within the limits of experimental error this relationship is the same as that for the combustion of sugar. The heat of combustion of sugar is 673,000 calories per mole, and since 1 mole of sugar gives off 6 moles of CO_2, one mg. of CO_2 is equal to 2.54 calories.

If, as BIALÆ has shown, a kilogram of lemons give off 180 mg. of CO_2 per day, then they also give off 180×2.54 calories or 457 calories of heat. One pound of lemons would give off 207 calories per day.

One car of lemons in storage contains 655 boxes of 50 lb. each or about 32,750 lb. Then 32,750 lb. $\times$ 207 or 6,779,250 calories of heat are given off per car per day at 59° F. Since 6,779,250 calories is the heat required to melt 187 lb. of ice, every 10.7 cars of stored fruit will require one ton of ice every 24 hours to remove the heat given off by the normal respiration.

BIALE and SHEPHERD (**3**) have shown that the respiration rate of normal lemons is approximately doubled by the respiratory gases given off by one lemon decaying with *Penicillium digitatum* when these gases are mixed with air and passed over the fruit at the rate of 3.6 liters per minute, a rate more than adequate to supply the oxygen for respiration of the fruit involved.

If it is assumed that this approaches a condition equal to that obtained for 1 per cent. green mold decay in a lemon storage room, then one additional ton of ice is going to be required by each 10.7 cars of lemons stored under these conditions.

Discussion

While the epinasty test has been known to be an indicator of the presence of ethylene, to the author's knowledge it has not been used to any extent commercially. Records of the California Fruit Growers Exchange indicate that the idea of using epinasty of tomatoes to measure ethylene in lemon storages existed as long as ten years ago. No way of growing tomato plants successfully in the low temperatures of the lemon storage was available at that time. In the present investigations it has served admirably as an accurate and simple test for ethylene.

One of the questions which will no doubt arise in the reader's mind is whether the carbon monoxide or some other of the automobile gases may not be responsible for the epinasty observed in experiments with these gases. CROCKER, ZIMMERMAN and HITCHCOCK (**4**) tested 38 different gases but found only five which produced epinastic effects and the minimum effective concentrations of those gases were as follows:

Ethylene	0.2 p.p.m. in air
Acetylene	250.0 p.p.m. in air
Propylene	1000.0 p.p.m. in air
Carbon monoxide	5000.0 p.p.m. in air

Butylene is not listed as affecting peas but is given as $\frac{1}{500,000}$ as effective as ethylene on tomatoes.

If there were as much acetylene as ethylene in the automobile exhaust gases it would be responsible for only $\frac{1}{1250}$ or about one-twelfth of one per cent. of the effect. KOBER and HAYHURST (13) list motor exhaust gases as containing about 9.3 per cent. carbon monoxide. This would be about 93,000 p.p.m. or about 52 times as much as ethylene; but since ethylene is 25,000 times as effective in producing epinasty, only about 0.2 of one per cent. of the effect would be due to carbon monoxide. It therefore becomes evident that carbon monoxide is of little consequence as compared to the ethylene in the automobile fumes.

The epinasty test for ethylene content in commercial storage rooms reported here is very simple and easily cared for after it is set up. It requires no technical skill except as is required to estimate the extent of epinasty in the pea plants in storage as compared with those kept as controls. In some locations in urban or other heavy traffic areas it may be difficult at times to find places to keep the control plants where they are not affected.

Two or three hours a week of a man's time is sufficient to keep tests going under most storage conditions.

Summary

1. This paper describes methods of using the epinasty reaction of pea seedlings for measuring small concentrations of ethylene in the exhaust fumes of automobile and in fruit storage rooms.

2. Data indicate a close relationship between the quantity of ethylene necessary to color lemons and that necessary to cause epinasty to pea seedlings.

3. The concentration of ethylene necessary to cause noticeable epinasty or lemon coloring has been found to be between 0.025 p.p.m. (1 part ethylene in 40 million) and 0.05 p.p.m. (1 part in 20 million) of air.

CALIFORNIA FRUIT GROWERS EXCHANGE
ONTARIO, CALIFORNIA

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RESPIRATION OF MOSAIC-INFECTED TOBACCO PLANTS

F. LYLE WYND

(WITH ONE FIGURE)

There has been reported in the literature a number of attempts to observe *in vitro* the respiration of virus preparations in an effort to detect direct evidence of a valid metabolic activity. EATON (**10**) studied manometrically the respiration of cultures of Staphylococcus lysed by bacteriophage and found a detectable amount of oxygen absorption and carbon dioxide production. On the other hand, BRONFENBRENNER (**2, 3**) BRONFENBRENNER and REICHERT (**4**), BACHMANN and WOHFIEL (**1**), WOHFIEL (**19**), PARKER and SMYTHE (**15**), and WYND and BRONFENBRENNER (**21**), using several types of extraordinarily sensitive procedures, were unable to observe any evidence of a respiratory metabolism by preparations of bacteriophage. PIRIE and HOLMES (**16**) reported that Agalactia virus absorbed large amounts of oxygen when suspended in a saline solution containing lactate, and these authors believed, therefore, that virus particles were living organisms.

The respiratory rates of green plants infected with virus have also received a considerable amount of study. BUNZEL (**5**) remarked that sugar beets stunted by the Curly-top disease suffered from a "fever." Later (**6**) he stated that a respiratory study of these "feverish" plants would be highly desirable in the effort to interpret the more exact nature of the disturbances which the disease produced. THUNG (**18**) reported that potatoes infected with leaf-roll eliminated more carbon dioxide than healthy tissue, although a study of his data shows that this was not always true. DUNLAP (**9**) studied the rates of carbon dioxide production of a number of species and found that the virus-affected plants exhibited an increased respiration in their younger tissues and a decreased rate in the older tissues. He observed the mosaic leaves of tobacco, tomato, pokeweed (*Phytolacca decandra*), cucumber, and raspberry, and yellows-affected leaves of peach, plum, aster, and ragweed (Ambrosia). The conclusions of DUNLAP (**9**) were criticized by CALDWELL (**7**) because the data were obtained from tissues respiring in an atmosphere of reduced oxygen content. Further, he doubted if efficient absorption of carbon dioxide was obtained under the condition of the experiment. He also believed that the period of the observation was too short. CALDWELL collected in barium hydroxide the carbon dioxide produced and titrated the remaining alkali after 48 hours. He found an increased carbon dioxide production by virus-infected tomato plants. LEMMON (**14**) made an extensive study of the comparative rates of metabolism of healthy and mosaic-infected tobacco leaves and found that the diseased leaves always had a lower respiratory rate than normal leaves. His data were expressed in terms of the fresh weight of excised discs of tissue. He further observed that discs of healthy tissue weighed only 85 per cent. as much as did the equal discs of diseased tissue.

The present work on the respiratory rates of healthy and mosaic-affected tobacco leaves was undertaken in an effort to explain the conflicting data on the respiration rates of virus-infected green plants, and to accumulate evidence on the nature of the substance responsible for the tobacco mosaic disease.

Procedure

Seeds of Burley tobacco were germinated in the greenhouse and transplanted into two-inch pots as soon as the plants were large anough to handle. As growth progressed they were transplanted into four-inch and finally into eight-inch pots. A great many more plants were grown than were needed for the experimental work in order that plants identical in appearance could be selected at the proper time. The plants were large enough for study by the first of May, nine weeks from the time of planting. When the plants had five well-developed leaves, the two lower leaves were removed, the lowest remaining leaf dsignated as number 1, and the higher leaves numbered consecutively. Several new leaves appeared during the course of the observations, and they were designated by consecutively higher numbers as soon as they reached a size large enough to be included in the experimental material.

A virus preparation was made by grinding the upper leaves of severely infected plants and squeezing the juice through cheesecloth. Inoculations were made by dipping a small pad of cheesecloth into this juice and gently rubbing the surface of the leaf. In all cases, only leaf 1 received the inoculation. At intervals of two or three days, three control plants and three inoculated plants were removed to the laboratory for study.

The rate of oxygen use was determined on a composite sample obtained by cutting three discs from each leaf, equally numbered from each of the three plants. The data therefore represent the total oxygen use of nine discs, three from a leaf of comparable age from each of three plants. The discs were cut by a sharp cork borer ten millimeters in diameter. Their oxygen consumption was determined at 25° C. by the Barcroft differential manometers. Since the data of LEMMON show that healthy discs weighed only 85 per cent. as much as those of diseased tissue, it is seen that the present procedure of comparing tissue samples having an equal area gives differences varying somewhat in magnitude from those which would have been obtained if equal weights had been used. Both of these methods of comparison are subject to error since neither the weight nor the area of tissue samples strictly represents the absolute amount of living protoplasm.

After the discs were removed from the leaf, the remaining tissue was finely ground in a mortar with purified quartz sand and the juice extracted by squeezing the pulp through several thicknesses of cheesecloth. Tests for the presence of the virus were made by the inoculation of young tobacco plants with this juice. HOLMES (**11**) has correctly pointed out that the inoculation of the press juice detects only an *infectious* concentration of

the virus, and not necessarily its first appearance. Determinations of reducing sugar, invertase, oxygenase, peroxidase, and catalase were also made on each of these juice samples and these data have been reported in a previous paper (**20**).

Experimental data

Examination of the data presented in table I shows the time necessary for the appearance of infectivity in various leaves of the plant after the inoculation of the lower leaf (number 1). Leaf 1 was infectious from the beginning of the experiment, as could be expected, since it received the inoculation. The virus left on the surface from the inoculation itself could have caused infection in this instance. Leaves 2 and 3 became infectious on the fourteenth day, leaf 4 on the eighth day. Leaves 5 and 6 were not yet present when the inoculation occurred, yet they appear to be among the

TABLE I

THE TIME NECESSARY FOR THE APPEARANCE OF VIRUS IN VARIOUS LEAVES OF TOBACCO PLANTS AFTER INOCULATION OF LEAF 1 (THE LOWEST) WITH VIRUS +, VIRUS PRESENT; −, VIRUS ABSENT; 0, LEAF NOT YET PRESENT

DAYS AFTER INOCULATION	LEAF 1	LEAF 2	LEAF 3	LEAF 4	LEAF 5	LEAF 6
2	+	−	−	−	0	0
4	+	−	−	−	0	0
6	+	+*	−	−	+	0
8	+	−	−	+	−	−
11	+	−	−	+	+	−
14	+	+	+	+	−	+
16	+	+	+	+	−	+
18	+	+	+	+	+	+
21	+	+	+	+	−	+

* This appears to have been due to the use of an infected plant since 8 days elapsed before the second leaf became infectious in all other experiments.

first tissues to become infectious. It seems probable that the upper tissues were the first to become infectious by means of newly formed virus material, even though the lowest leaf had received the inoculation. Under the growing conditions of the present experiments, the entire plant became infectious at about the fourteenth day. The duration of this period would vary with the growing conditions, and with the age and vitality of the plant.

The cubic millimeters of oxygen used by leaves from normal and from inoculated plants are presented in table II. These rates of oxygen use by leaves from inoculated plants, calculated as the percentage of the use by leaves of similar age from normal plants, are presented in table III. It is difficult to obtain exactly equal samples of leaf tissue by the method described above. For instance, younger leaves differing only slightly in their stage of development may vary greatly in the extent of their rolling and evenness of their surfaces, and since equal areas are used as the basis for

TABLE II

THE USE OF OXYGEN IN CUBIC MILLIMETERS BY NINE EXCISED DISCS FROM LEAVES OF NORMAL AND INOCULATED TOBACCO PLANTS. N, LEAVES

TABLE II

THE USE OF OXYGEN IN CUBIC MILLIMETERS BY NINE EXCISED DISCS FROM LEAVES OF NORMAL AND INOCULATED TOBACCO PLANTS. N, LEAVES FROM NORMAL PLANTS; IN, LEAVES FROM INOCULATED PLANTS

Time	Second day						Fourth day					
	Leaf 1		Leaf 2		Leaf 3		Leaf 1		Leaf 2		Leaf 3	
	N	In	N	In	N	In	N	In	N	In	N	In
min.	*cu. mm.*	*cu. mm.*	*cu. mm.*	*cu. mm.*	*cu. mm.*	*cu. mm.*	*cu. mm.*	*cu. mm.*	*cu. mm.*	*cu. mm.*	*cu. mm.*	*cu. mm.*
15	11.0	10.9	8.7	11.1	13.9	12.9	16.1	21.4	.....	19.5	15.0	18.1
30	22.2	23.4	17.7	22.0	27.6	26.8	27.4	37.6	.....	33.6	28.0	32.6
45	33.0	34.3	27.4	33.2	40.5	40.0	36.2	49.6	.....	46.0	40.8	45.5
60	44.4	45.5	36.8	44.0	54.2	53.0	.....	.....	.....	.....	.....	.....
	Sixth day						Eighth day					
15	6.9	8.6	8.1	9.3	14.4	13.8	8.8	8.8	8.8	7.4	12.2	11.2
30	14.8	17.5	16.4	18.6	28.6	24.7	17.0	15.9	16.7	14.4	24.5	22.4
45	22.2	26.7	25.0	28.4	43.0	37.5	25.2	23.8	25.0	22.1	36.8	34.7
60	30.6	36.0	34.2	37.8	57.6	49.8	33.9	31.0	33.4	29.5	49.5	47.0
	Eleventh day						Fourteenth day					
	Leaf 1		Leaf 2		Leaf 6		Leaf 1		Leaf 2		Leaf 6	
15	7.2	8.4	9.5	7.9	11.8	10.7	7.3	9.6	10.2	8.6	12.9	11.5
30	15.0	16.2	16.7	15.5	22.3	21.5	15.7	18.4	21.9	17.2	27.0	23.2
45	25.8	24.2	25.2	23.5	33.1	32.2	25.2	27.0	30.6	26.8	40.7	35.8
60	30.6	31.9	31.2	30.7	43.3	42.2	34.1	35.4	39.4	35.8	53.9	48.8
	Sixteenth day						Eighteenth day					
15	10.5	10.9	11.6	10.6	13.4	6.0	8.8	6.1	9.1	6.9	9.3	9.4
30	20.5	21.5	21.8	20.4	26.8	13.2	17.6	13.8	16.3	14.0	19.2	19.1
45	29.7	31.7	31.0	30.7	39.3	20.0	27.1	22.1	25.2	22.0	28.6	28.8
60	39.5	41.3	39.8	41.8	51.8	30.2	35.6	29.8	35.6	29.3	38.4	38.3
	Twenty-first day											
15	8.6	8.4	8.8	7.7	10.3	7.4						
30	16.6	17.1	16.9	15.6	20.1	15.2						
45	25.2	25.8	24.6	22.7	29.4	22.6						
60	33.4	33.7	32.5	30.4	37.8	29.4						

TABLE III

THE USE OF OXYGEN DURING THE TIME INDICATED BY LEAVES FROM INOCULATED TOBACCO PLANTS CALCULATED AS PERCENTAGE OF THE OXYGEN USE BY LEAVES FROM THE NORMAL PLANTS

Days after inoculation	Oxygen use in 15 minutes				Oxygen use in 30 minutes			
	Leaf 1	Leaf 2	Leaf 3	Leaf 6	Leaf 1	Leaf 2	Leaf 3	Leaf 6
	%	%	%	%	%	%	%	%
2	99.3	127.6	92.7	.....	105.3	125.7	97.3	.....
4	132.9	.....	120.6	.....	137.5	.....	116.5	.....
6	124.6	113.0	96.0	.....	118.3	113.5	86.4	.....
8	100.0	84.4	91.7	.....	93.5	86.3	91.3	.....
11	116.7	83.3	.....	90.8	107.9	92.8	.....	96.5
14	131.8	76.8	.....	89.3	117.8	78.7	.....	85.7
16	103.9	91.5	.....	44.8	105.0	93.8	.....	49.3
18	69.3	75.9	.....	101.3	78.4	86.0	.....	99.7
21	97.7	87.5	.....	71.9	103.0	92.5	.....	75.6
	Oxygen use in 45 minutes				Oxygen use in 60 minutes			
2	103.8	121.4	99.0	.....	102.5	119.8	97.9	.....
4	137.7	.....	116.5	.....	.....	.....	.....	.....
6	121.5	113.8	87.3	.....	117.6	110.5	86.4	.....
8	94.3	88.5	94.4	.....	91.4	88.3	94.9	.....
11	93.5	93.4	.....	97.3	104.2	98.4	.....	97.4
14	107.5	87.5	.....	87.9	103.8	90.8	.....	90.5
16	106.5	99.2	.....	50.9	104.5	105.0	.....	58.3
18	81.7	87.5	.....	100.7	83.7	82.3	.....	99.7
21	102.6	92.3	.....	77.0	100.9	93.5	.....	77.7

comparison, erroneous results are sometimes obtained. Therefore, the data are presented in considerable detail, so that the reader may judge the validity of the conclusions presented below.

A survey of the data shows the following relationships to exist. At the time the experiment was begun, there were three leaves large enough for study. On the second day after inoculation of leaf 1 (the lower), none of the leaves showed any significant change in the rate of oxygen use as compared to the rates of leaves from normal plants. An exception in the case of leaf 2 is probably an error because the observed rate of leaf 2 from normal plants used for comparison was too low. From table II, it is seen that leaf 2 of normal plants has a value of only 8.7, although it is obvious that it should have been about 11.0. This is a reasonable assumption because the data presented in the present paper and certain other data obtained in the writer's laboratory indicate that the rate of oxygen use increases progressively in leaves from the upper portion of the tobacco plant.

On the fourth day after inoculation, all three leaves showed an abrupt increase in oxygen use. From figure 1 it is seen that leaf 1 showed the greatest increase, and that this increase was progressively less in the upper leaves. On the sixth day, this increase was yet apparent, although it had diminished in magnitude. By the eighth day, however, the leaves from the inoculated plants were either less than those of normal plants, or were ap-

proaching the control value. In view of subsequent determinations, presented in table I, it is possible that these values are too low. However, the fact that each determination was made on a composite sample from three plants indicated that the lessened rate was approximately that of its observed value. After the eleventh day, the respiration rates became progressively less, although these rates of diminution became less rapid with time. This lessened rate could not have been due to a mere advance in maturity since leaf 6 also had a lessened rate even though it was the youngest leaf.

Six manometers were available which permitted the comparison of only three sets of leaves. By the eleventh day, two additional leaves, numbered 5 and 6 respectively, were large enough for study, and since the terminal

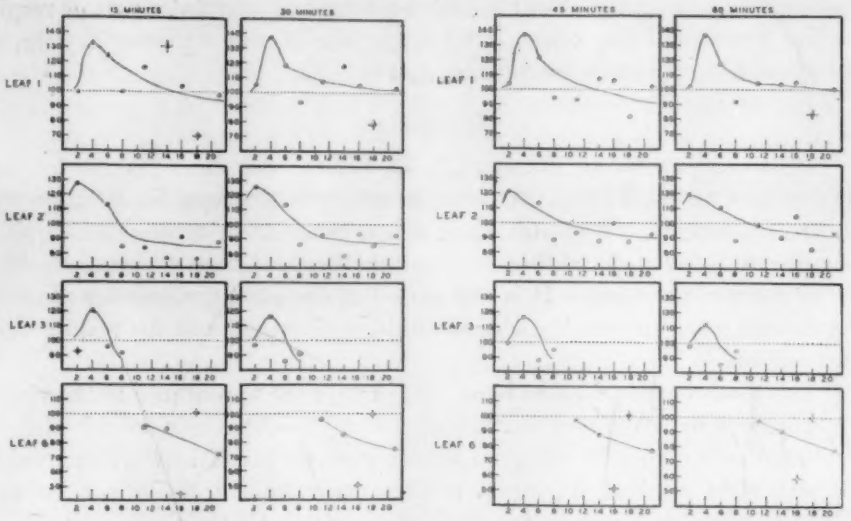

FIG. 1. The oxygen consumption of 9 discs of tissue excised from the leaves of tobacco plants inoculated with mosaic virus. The ordinates represent the data in terms of percentage of the oxygen use by discs from normal leaves of similar age. The abscissae represent the days elapsed since inoculation.

part of the plant is apparently especially concerned with the production of virus, the observations on the originally terminal leaf 3 were discontinued in favor of leaf 6. Even before leaf 6 appeared, infected plants exhibited a generally lessened respiration rate and this characteristic occurs from the very beginning in the newly formed leaves. It is interesting to note that the rates of oxygen use by leaf 6 appear to fall on the latter part of the curve typical for the older leaves which were on the plant from the time of inoculation.

A comparison of respiratory rates presented in figure 1 with the data in table I shows that the times necessary for the appearance of infectivity in the various leaves are not at all related to the periods of disturbed metabolism indicated by the increased rate of oxygen use. The peak of the disturbed oxygen use occurred at about the fourth day *for all leaves*. At this

time, leaf 1 contained the virus, since it had received the inoculation, but the upper leaves had not yet developed the power to infect young normal plants. Unfortunately, the lack of equipment prevented a study of the oxygen consumption in all leaves, and consequently, the rates of leaves 4 and 5 are unknown.

The curves showing the amount of oxygen used in 60 minutes are probably the most authentic since the magnitude of the manometer reading was greater. This set of figures shows that the inoculated lower leaf regained an approximately normal rate of respiration after the eleventh day, even though it contained the active virus. Leaf 2, at about the same time, had assumed a rate less than normal. The study of leaf 3 was discontinued on the eighth day in favor of leaf 6, and this later leaf assumed a rate of respiration lower than any other. The magnitude of this decrease in rate is progressively greater in the younger leaves.

Discussion

It has been shown by HOLMES (**11, 12, 13**) and SAMUEL (**17**) and by the review by CRAFTS (**8**) that the virus spreads from the point of infection by a comparatively slow movement from cell to cell. After three or four days, the phloem is invaded and then in a remarkably short time the virus reaches other parts of the plant. It is this period of dispersal through the phloem tissue that seems to coincide with the sudden disturbance of the respiratory metabolism.

This disturbance preceded by about ten days the appearance of the virus in infectious concentration throughout the plant. Although the initial disturbance in metabolism occurred simultaneously in all leaves, the newly formed virus attained infectious concentration first in the upper leaves. These also are the leaves having the greater metabolic activity.

If the physiological disturbances indicated by the change in the rates of oxygen use were manifestations of the activity of the virus particles *per se*, then these changes should accompany the increase in the number of these particles. On the other hand, if the mosaic disease is caused by some chemical product of abnormal metabolism, which like the bacteriophage, could itself initiate a series of reactions which would produce new virus material during some subsequent process, one might then expect to detect a disturbed metabolism before the appearance of any considerable amount of new virus substance. Although observations of the latter type do not demonstrate conclusively the validity of such an inference, they do indicate that the increased rate of respiration could not be due to the metabolism of the virus particles themselves.

It is apparent that the data on the respiration of mosaic infected plants published by DUNLAP and by LEMMON are not necessarily contradictory since diseased plants have either an increased or decreased rate depending on the time which has elapsed since infection.

Summary

1. The rate of oxygen use by leaves from mosaic-affected tobacco plants is greatly increased by the fourth day after a lower leaf was inoculated.

2. The period of stimulated oxygen use precedes by approximately ten days the general appearance of the mosaic agent in infectious concentration.

3. Although the disturbed metabolism indicated by the increased rate of oxygen use occurs simultaneously throughout the plant, the appearance of infectious concentration of new virus material occurs first in the upper leaves, excepting, of course, the leaf which had received the inoculation. These leaves have a higher initial rate of metabolism.

4. By the time infectious concentrations of virus appear in the upper leaves, the rates of oxygen use by these leaves are always less than that of normal leaves.

5. Since infectious material appears only subsequent to a disturbed metabolism, it is probable that the observed metabolic changes are cellular in nature and do not depend on any metabolic activity of the virus material.

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UNIVERSITY OF ILLINOIS
URBANA, ILLINOIS

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RIDGES AND SECTORS INDUCED IN THE RIND OF CITRUS FRUITS BY FUMIGATION WTH HYDROCYANIC ACID[1]

WALTON B. SINCLAIR AND DAVID L. LINDGREN

(WITH FOUR FIGURES)

The fumigation of citrus trees with hydrocyanic acid (HCN) for pest control, at certain times of year and under certain environmental conditions, produces irregular and excessive growth of the outer peel (flavedo) of the fruit (**3**). This abnormality is commonly known as "ridging" or "coxcombing." That ridging occurs in some groves to a greater extent than in others has been generally recognized, but growers and field men have until recently been unable to trace the cause.

In some fruits, the growth may appear as a single smooth ridge in the form of a longitudinal sector; other fruits may have several longitudinal sectors of equal or unequal width (fig. 1). The amount of ridging varies considerably. The ridges may extend the full length of the fruit, from the calyx to the stylar end, or they may be small and irregularly placed over the entire fruit surface.

Deformations or variations on fruit surfaces occur naturally, to a limited degree, as bud variants in all varieties of citrus (**2**). SHAMEL and his coworkers (**4**) have studied these bud variations intensively from a commercial standpoint, identifying them largely by their appearance on the fruit. They have shown that individual fruit variations from limbs on given trees can be transmitted by means of bud propagation. They state: "The degree of inherent stability of the progenies has been found to be about the same as the condition of uniformity in the characteristics of the parent bud variations. If the fruit or foliage of these limb or tree variations was uniform their progenies have been found to be uniform; on the other hand, if there was a marked variability of the fruit or leaves in the parent limb variations the progenies have usually been relatively variable."

The purpose of this paper is to show that certain individual fruit variations, similar to those ordinarily transmitted and produced by means of bud propagation, can, under certain conditions, be induced by fumigation with HCN.

It was decided to determine the amount of ridging on the fruit of trees fumigated at different seasons of the year. Groves in which some trees could be fumigated at various times of year and others, to serve as controls, could go without fumigation, were selected for this investigation. As citrus is grown in southern California in both coastal and inland districts, experimental plots were located in commercial groves in both of these areas. For more than two years, extensive counts of the ridged fruits in the different groves were made. The results of the experiments, showing the

[1] Paper no. 470, University of California Citrus Experiment Station, Riverside, California.

relation between the time of year of the fumigation and the amount of sectors and ridges formed on the fruits, are of sufficient interest and importance to warrant publication at this time.

Experimental procedure

In southern California, citrus trees are fumigated with HCN for pest control from mid-July to early spring, a period of approximately eight months in which commercial fumigators can operate. During the months of May, June, and part of July fumigation is discontinued as fruits are then more susceptible to injury than at other times of year. For a short period in the fall, also, usually in October, the trees are rather susceptible and fumigation is suspended. The data reported herein were obtained from

FIG. 1. Ridged Valencia oranges and a normal fruit from trees which had 31.4 per cent. of their fruits affected (Grove 2, table I).

trees fumigated in November, December, January, and February, preliminary experiments having indicated that trees fumigated during January and February were the ones on which ridging of the fruits was most likely to occur.

The experiments were so planned that the fumigated trees were scattered throughout the plots. (Figure 2 illustrates the type of plot design.) Fumigation of certain trees in this manner is commonly referred to in fumigation practice as "spot fumigation," in contrast with the usual method, in which all the trees of an orchard are fumigated. A distinct advantage in fumigating scattered trees is that untreated trees (controls) are in close proximity to adjacent treated trees and receive similar cultural treatment. This condition partially equalizes any possible effects on ridging due to variations in soil, fertilization, irrigation, etc. The concentration of HCN

used in each fumigation varied from 20 ml. to 28 ml. per unit. A unit in citrus fumigation is approximately 100 cu. ft. for the average-sized tree.

The amount of fruit that was ridged was determined by counting 200 fruits, at random, around each of the trees, on inside and outside positions, and recording the number affected. The percentages were calculated on a basis of 200 fruits per tree.

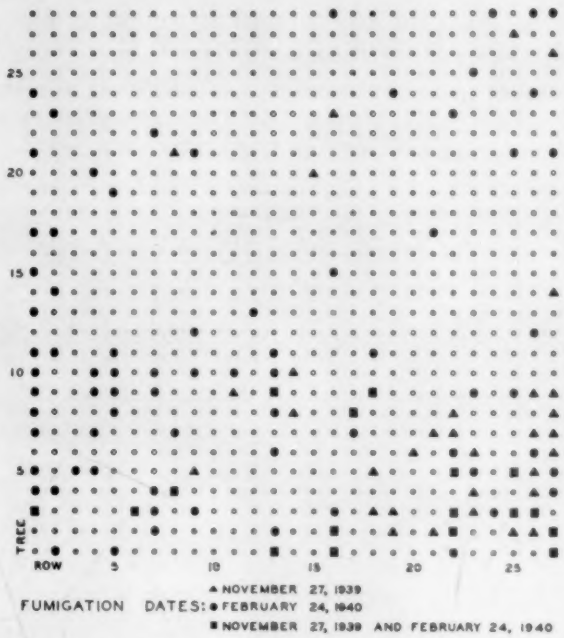

FIG. 2. Diagram of an experimental plot, showing location of trees fumigated at different times of year.

Results

The results shown in table I are typical of those obtained in this investigation. Data are reported for navel and Valencia oranges, grapefruit, and lemons and are so arranged that the average percentages of ridged fruits on trees receiving the different treatments in each grove can be compared. As each grove represents a different experiment in a different location, a brief description of the results in each grove is given.

Some very interesting comparisons can be made from the data reported from grove 1 (table I). Only a small percentage (0.1 per cent.) of the fruits counted on the unfumigated trees (controls) were ridged. Of fruits counted December 11, 1940, on 24 trees fumigated February 24, 1940, an average of 36 per cent. had ridges and sectors. Counts of fruits were also made December 11, 1940, on 18 trees that had been fumigated November 27, 1939. The average amount of ridged fruits on these trees was the same as that on the controls (0.1 per cent.). But when 13 trees that had been

TABLE I

PERCENTAGES OF CITRUS FRUITS HAVING RIDGES AND SECTORS IN THE RIND, FROM TREES FUMIGATED WITH HCN AT DIFFERENT TIMES OF YEAR AND FROM UNFUMIGATED TREES

GROVE	VARIETY OF CITRUS	NUMBER OF TREES	HCN FUMIGATION		DATE OF SAMPLING	MEAN PERCENTAGE OF FRUITS RIDGED†
			HCN PER UNIT*	DATE		
			ml.			%
		26 (Control)	...	.....	Dec. 11, 1940	0.1
		24	24	Feb. 24, 1940	Dec. 11, 1940	36.0
1	Navel orange	18	20	Nov. 27, 1939	Dec. 11, 1940	0.1
		13‡	20 24	Nov. 27, 1939 Feb. 24, 1940	Dec. 11, 1940	36.6
		6 (Control)	....	.....	Jan. 3, 1941	0.1
2	Navel orange	8	20	Dec. 14, 1939	Jan. 3, 1941	0.8
		8	22	Feb. 21, 1940	Jan. 3, 1941	24.3
		8 (Control)	....	.....	Jan. 3, 1941	0.1
	Valencia orange	7	20	Dec. 14, 1939	Jan. 3, 1941	0.2
		8	22	Feb. 21, 1940	Jan. 3, 1941	31.4
3	Navel orange	11 (Control)	....	.....	Aug. 14, 1941	0.0
		11	24	Feb. 4, 1941	Aug. 14, 1941	28.3
4	Grapefruit	21 (Control)	....	.....	Jan. 3, 1941	0.2
		25	24	Feb. 16, 1940	Jan. 3, 1941	22.1
5	Lemon	8 (Control)	....	.....	Mar. 11, 1941	0.2
		14	28	Feb. 26, 1940	Mar. 11, 1941	40.1

* A unit in citrus fumigation is approximately 100 cu. ft. for the average-sized tree.
† Percentages based on counts, at random, of 200 fruits per tree.
‡ These trees were fumigated twice, once on Nov. 27, 1939, and again on Feb. 24, 1940.

fumigated on November 27, 1939, were fumigated a second time, on February 24, 1940, the percentage of ridged fruits produced was 36.6. These experimental data therefore demonstrate that navel orange trees can be fumigated in November without increasing the amount of ridged fruit.

Grove 2 consisted of both navel and Valencia orange varieties, planted alternately in the rows. Six trees served as controls for the navel plot, and 8 trees served as controls for the Valencia plot. Each of the control plots contained 0.1 per cent. ridged fruits. In the navel plot, two sets of 8 trees each were fumigated, one on December 14, 1939, and the other, February 21, 1940. In the Valencia plot, 7 trees were fumigated December 14, 1939, and 8 trees were fumigated February 21, 1940. In the two groups of trees that were fumigated December 14, 1939, the navels had 0.8 per cent. and the Valencias 0.2 per cent. ridged fruits. In the two groups of trees that were fumigated February 21, 1940, the navels had 24.3 per cent. and the Valencias 31.4 per cent. ridged fruits. In this particular grove, it can be seen that the ridging of the fruit on the trees fumigated in February was vastly significant, whereas the ridging of the fruit on the trees fumigated in December was of the same order of magnitude as that of the controls.

The trees in grove 3 were fumigated February 4, 1941, and the fruit was counted for the amount of ridging on August 14, 1941. As these trees were of the navel variety, the size of the fruit at the date of counting ranged from ½ to 1 inch in diameter. The fumigated trees showed an average of 28.3 per cent. of ridged fruit, while the control trees showed none at all.

Some experimental data were obtained from grapefruit trees (grove 4) fumigated February 16, 1940. The mean percentage of ridged fruit on 25 fumigated trees was 22.1, and the mean percentage of ridged fruit on the 21 control trees was 0.2.

Lemon trees (grove 5) that were fumigated February 26, 1940, fruits of which were counted on March 11, 1941, showed 40.1 per cent. ridged fruits. The untreated trees in this grove had 0.2 per cent. of their fruits affected.

Discussion

The data of table I show that fumigation of citrus trees with HCN, when the fruit was in a certain stage of bud development, resulted in the formation of sectors and ridges in the outer peel of the fruits of navel and Valencia orange, grapefruit, and lemon varieties. The time of year in which ridging will occur varies somewhat with the different varieties of citrus. In the navel and Valencia orange and in the grapefruit varieties, the highest percentage of fruits affected was from the trees fumigated in the month of February, although in some groves extreme ridging of the fruits has been observed to occur on trees fumigated the latter part of January. Extensive field observations have shown that fruits from lemon trees become severely affected if the trees are fumigated any time from late January to April, inclusive. Fruit buds of lemon trees develop at different

times of year, but the largest setting of fruit occurs in the spring over a prolonged period.

The ridging of citrus fruits may therefore be said to depend upon the stage of development of the fruit buds at the time the trees are fumigated. Variation in the time and rate of bud development in different groves and in different years indicates a certain variation in the time of year in which fumigation will cause the development of ridging in the fruit.

The form of the rind thickenings is very suggestive of that found in the well-known sectorial chimeras which are frequent in citrus (**2**). It is possible that the change caused by HCN often affects one or more cells in the developing ovary before cell division is ended. This change may be trans-

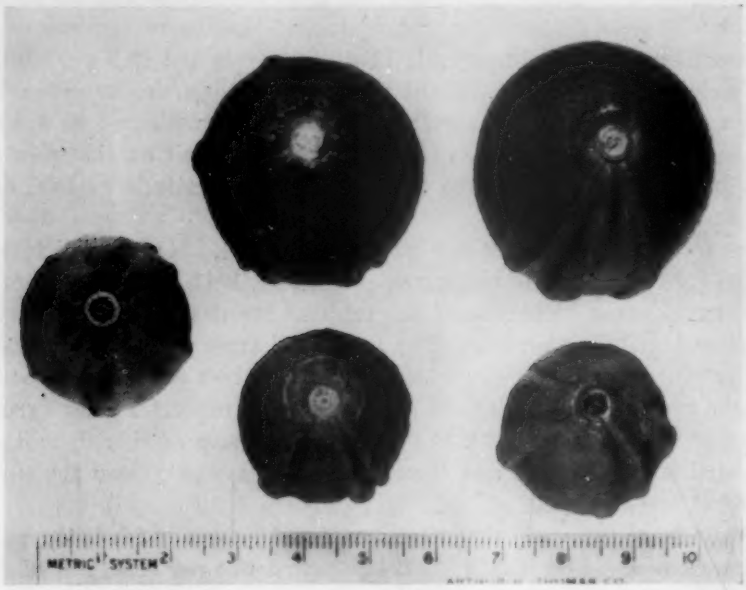

FIG. 3. Ridges on small, immature navel oranges selected from trees which had 28.3 per cent. of their fruits affected (Grove 3, table I).

mitted in the process of mitotic cell division and result in the formation of sectors originating from one cell or from a relatively small number of cells. Whether rind thickening is due to an increase in cell number or in cell size has not been determined. It may be that HCN causes a genetic change, such as a doubling of the number of chromosomes. Sectors of modified tissue appear in plants after treatment with colchicine, owing to the presence of tetraploid sectors, as in Lilium (**1**).

Just what effect climatic conditions have on the severity of the ridging of citrus fruits when fumigated with HCN is not known. It has been suggested by some workers that a warm, mild winter contributes to the ridging of fruits, but no experimental data are available on this point. Climatic and seasonal changes undoubtedly affect the time and rate of bud develop-

ment; but an important factor in producing this phenomenon in fruits appears to be the definite stage of bud development at the time of fumigation. *If the trees are fumigated prior to or subsequent to this particular and definite stage in development of the bud, only the natural percentage (0.1 to 1.5) of ridged fruits will result.* According to our experimental results and field observations, HCN affects only the fruit that is in the bud stage of development at the time of fumigation; the fumigation of the trees after the fruit has formed does not increase the amount of ridged fruit.

The effect of the HCN does not carry over to the following year. As an illustration, trees that were fumigated on February 26, 1940, had an average of 25 per cent. of ridged fruit for that crop. These trees were not fumigated in February, 1941, and the green fruit from the buds of the suc-

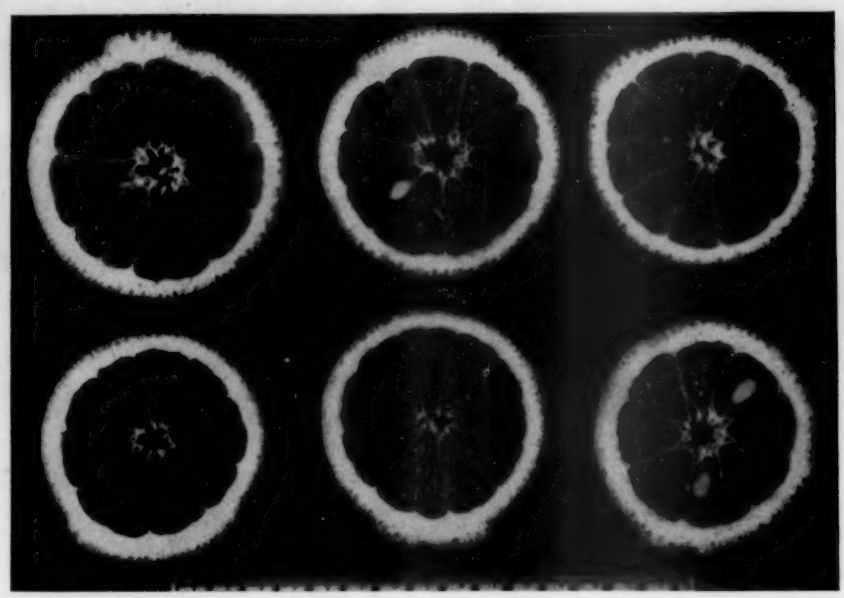

FIG. 4. Cross sections of mature Valencia oranges, showing that the location of ridges on the fruit surface is in no way correlated with the arrangement of fruit segments.

ceeding crop showed no ridging. Observations of this kind were made on many groves in which both crops of fruit, mature and immature (green), were on the trees at the same time.

The appearance of ridges and sectors after fumigation is not correlated with size of the fruit. Ridging is about equally distributed, occurring on fruit of all sizes on the tree. In fact, the ridges appear on the very small, immature fruit (fig. 3) and they persist throughout its growth and maturation. This phenomenon appears to be confined solely to the outer peel, the flavedo (fig. 4). The physical appearance of the albedo, and of segments of affected fruit, is normal in every respect. The location of the ridges on the fruit surface is not in any way correlated with the arrangement of the segments inside the fruit (fig. 4).

Trees in a given grove, fumigated at the same time, varied as much as 100 per cent. in the number of fruits having ridges and sectors. When the amount of affected fruit on the fumigated trees was compared with that on the controls, the least amount of ridged fruit on the fumigated trees was, in every instance, more than ten times the greatest amount on the control trees. In accord with such experimental results, it is reasonable to conclude that the fumigated trees had a significantly greater amount of ridged fruits than the controls.

Conclusions

The fumigation of citrus trees, when the fruit buds are in a certain stage of development, induces ridges and sectors in the outer peel (flavedo) of the fruit.

In the navel orange, Valencia orange, and grapefruit varieties, fumigation of trees in February produced the greatest amount of ridged fruits, but in some years, extreme ridging of the fruits occurred on trees fumigated the latter part of January.

In the lemon variety, trees fumigated in February produced the highest percentages of fruits with ridges and sectors, but extensive field observations have shown that fruits of lemon trees fumigated from late January to April, inclusive, were severely affected.

Variation in the time and rate of bud development of citrus trees in different groves and in different years indicates a definite variation in the time of the year in which ridging will be produced in the fruit by fumigation. The development of ridges and sectors on citrus fruits depends mainly upon the stage of development of the buds at the time the trees are fumigated.

UNIVERSITY OF CALIFORNIA
CITRUS EXPERIMENT STATION
RIVERSIDE, CALIFORNIA

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OXIDATION-REDUCTION POTENTIALS OF THE TISSUE FLUID OF CABBAGE[1]

ELLIS AIROLA AND JOHN W. CRIST

(WITH TWO FIGURES)

Introduction

It has been stated (**2**) "that oxidation-reduction systems play so intimate and so essential a part in living organisms that life itself may be defined as a continuous oxidation-reduction reaction."

CANNAN (**1**), in 1926, investigated the electrode potentials of extracts obtained from the green shoots of *Mercurialis perennis*. Work of this kind, with higher plants—making use of the electrometric method—has not been extensive since the appearance of his publication. Additional effort in this field of investigation seemed to be warranted.

Materials and methods

The plant selected for use was cabbage (*Brassica oleracea*), in three of its leading commercial varieties: Ferry's Round Dutch, Large Wakefield, Early Jersey Wakefield.

The seeds for each successive lot of plants were germinated in moist sand. The seedlings, after emergence of the first leaves, were transplanted to four-inch pots. The soil of the pots was a homogeneous mixture of two-thirds compost and one-third sand. The pots, while the plants grew to the size required for sampling, were kept buried in moist sand on a greenhouse bench, and the percentage of moisture in them held essentially constant and at the optimum for the plants' need.

SAMPLING

When they had reached the desired size, the plants were cut off just above the soil surface; placed with their cut ends in water, and taken to the laboratory, where the leaves were quickly removed. Selection was made only of whole, well or fully developed, leaves, exclusive of their petioles, for the extraction of the tissue fluid. The size of the sample approximated 20 grams.

EXTRACTION

The selected leaves were put at once into a beaker and covered with diethyl ether. The time for their plasmolysis was a period that ended just before the cell sap began to exude. This period was found to vary with the ages of the leaves, and, from preliminary tests, was observed to be around thirty seconds for the younger leaves and one minute for the older ones. The plasmolyzed leaves were removed from the beaker; allowed to dry off on paper toweling; were wrapped in a prepared canvas cloth; placed in a

[1] Journal Article no. 593 (n.s.), Michigan Experiment Station.

nickel-plated brass cylinder; and extracted by means of an hydraulic press at a uniform pressure of 800 lb. per sq. in., with a uniform period of drainage of 45 seconds. The cylinder was of such design that the expressed juice could be drawn off into a pipette as rapidly as it appeared. This reduced the possibility of its oxidation from exposure to the air.

Instrumental measurements

The pH of the extracted juice was determined immediately by means of a Cameron (glass electrode) hydrogen-ion meter. The sample was then transferred to the oxidation-reduction cell, containing a glass-tube inlet through which bubbling oxygen-free nitrogen was admitted. Two platinum electrodes, in addition to the half-cell, dipped into the extract.

Preliminary tests showed that the platinum electrodes required from 4 to 6 minutes to come to equilibrium with the surrounding solution. This time was used to adjust the current of the amplifier (Leeds & Northrup, no. 7673, Thermionic) and also that of the standard cell, in the potentiometric system, to zero. With these zero points attained, the E.M.F. leads from the half-cell and one of the platinum electrodes were connected through the amplifier and the first reading taken. A duplicated reading was then made with the second, instead of the first, electrode connected in the circuit. These two readings, which in nearly all instances were less than three millivolts apart, were averaged. This average, after reference to a normal hydrogen electrode, was taken to be the oxidation-reduction potential of the extract.

Treatment of electrodes

Several methods for cleaning and restoring the electrodes were tried. Of these the most satisfactory way proved to be their submersion in fusing potassium acid sulphate, from which they were afterwards dissolved free in distilled water, and then let stand in distilled water until the next set of measurements was to be made.

Results

Relationship between pH and Eh values

Three separate sets of determinations, aimed to discover this relationship, were completed. The plants were of the Ferry Round Dutch variety and were respectively 43, 45, and 46 days old for the three tests.

The manner of testing the extracted sample was first to determine its pH value, and then its Eh value. Following this, it was returned to the pH receptacle and the desired amount of 0.1 N HCl added to it, whereupon its pH and Eh values were redetermined. This process was continued, with successive introductions of added amounts of the acid. The data appear in table I.

From inspection of table I the existence of a systematic relationship between pH and Eh seems to be evident. This relationship in the tests

TABLE I

EFFECT OF pH ON Eh (IN MILLIVOLTS) OF CELL SAP

Test 1		Test 2		Test 3	
pH	*Eh*	*pH*	*Eh*	*pH*	*Eh*
5.80*	+453*	5.78*	+471*	5.82*	+456*
4.30	+504	4.30	+520	4.85	+496
3.70	+527	3.80	+534	4.53	+504
3.30	+549	3.40	+548	3.50	+538
2.30	+587	3.10	+570	3.08	+555
1.70	+629	2.50	+581	2.80	+562
1.45	+660	1.97	+626	2.35	+610
1.20	+678	1.60	+662	2.05	+615
.....	.....	1.39	+681	.....	.....
.....	.....	1.29	+691	.....	.....

* Original extract.

proved to be curvilinear in character. The curve of best fit in each test was parabolic in type. When the measurements of all three of the tests were combined, and plotted, the result shown in figure 1 was obtained. The equation of the fitted curve is: $Y = 799.96 - 103.761\,X + 8.0937\,X^2$.

Leaf position and Eh values

It became a matter of interest to determine whether or not the oxidation-reduction potentials of the tissue fluid varied significantly within the plant

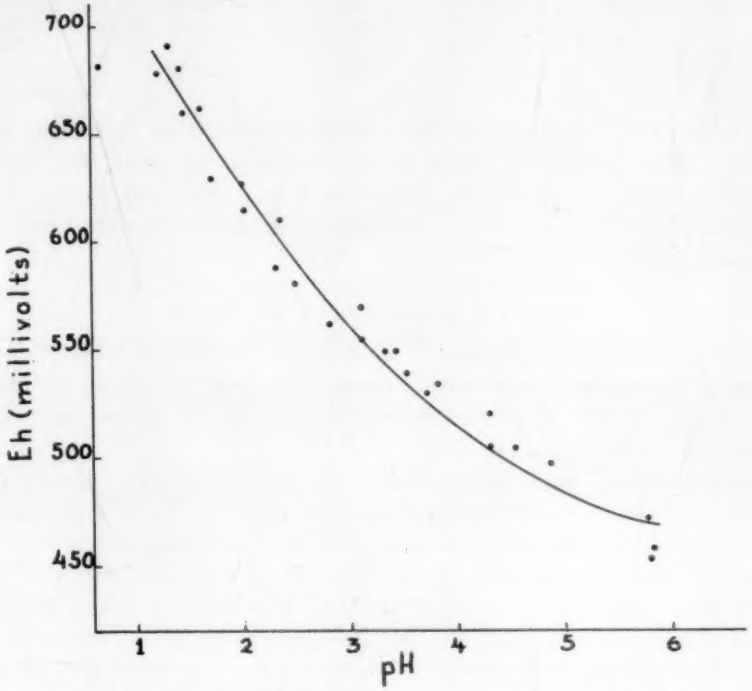

FIG. 1. Relationship between pH and Eh values of tissue fluid.

itself. Tests for this were made with respect to the positions of the leaves on the stem, for the Early Jersey Wakefield variety of cabbage.

Three such tests were completed. The plants were respectively 42, 44, and 46 days old, and were in the stage of each having 8 or 9 leaves. In each case, 9 plants, of uniform size and vigor, were selected and used. From these 9 plants the second leaves (ones next below emerging leaves) were taken and combined for use as a sample. The same procedure was followed for the third, fourth, fifth, and sixth orders of leaves, down the stem. The data appear in table II.

These tests (data in table II), based upon leaf-position, were essentially a consideration of the ages of the leaves. The sampling in each case ranged, through a series of grouped specimens, from juvenile, to virtually mature, to more or less senile leaves.

The data give some indication of systematic variance. The Eh values appear to increase with the development of the young leaf; reach maxima

TABLE II

Eh VALUES (IN MILLIVOLTS) FOR CELL SAP FROM DIFFERENT ORDERS OF LEAVES

ORDER OF LEAVES	TEST 1	TEST 2	TEST 3	MEAN
	Eh	*Eh*	*Eh*	*Eh*
2nd	+439	+453	+455	+449
3rd	+464	+469	+462	+465
4th	+453	+460	+469	+461
5th	+450	+460	+461	+457
6th	+447	+458	+450	+452

(3rd or 4th leaf), and beyond this tend to decrease with increasing age of the leaf. The differences, while mostly consistent in order, are, however, small in magnitude, and mostly insignificant in a statistical sense. Assuming them to be real, many more observations would be required certainly to establish significance.

EFFECT OF HARDENING ON Eh VALUES

It was of interest to ascertain whether or not the oxidation-reduction system of the cabbage plant would prove to be sensitive in its equilibrium to a change in the plant's environment. Exposure to low temperature (hardening) was chosen for trial.

One experiment was conducted with plants of the Ferry's Round Dutch variety; three with Ferry's Large Wakefield variety. In each instance, the plants were grown, as heretofore described, to a size adequate for use. At this point, which varied from 44 days for Ferry's Round Dutch to 35, 43, and 37 days respectively in the three tests with Ferry's Large Wakefield, the lot of plants was divided into two groups of selected and equal size and vigor. One lot was to be subjected to hardening, the other not (left continuously in the greenhouse).

PROCEDURE FOR HARDENING.—The lot of plants to be hardened was removed each day at 6 P.M. from the greenhouse to a cold storage room with a regulated temperature of 7° to 8.5° C. It was left there until 6 A.M. (a period of 12 hours), and then shifted back into the greenhouse. This was repeated for 6 consecutive nights.

Sample plants, for laboratory use, were taken each morning from each of the two lots between 9 and 10 A.M. The period from 6 A.M. to 9 A.M. sufficed for a return of the plants in the treated lot to the higher temperature of the greenhouse (16° to 22° C.).

The type of the results for the four experiments was essentially the same. It suffices, therefore, to present only the result from one experiment—that

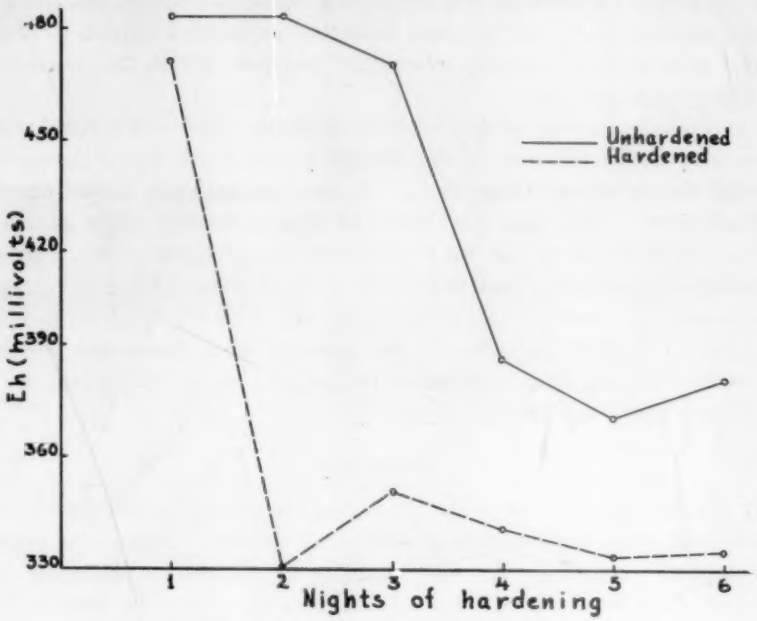

FIG. 2. Effect of hardening on Eh values of tissue fluid.

for Ferry's Large Wakefield variety—conducted in June, 1941. It is given by figure 2.

A decline in the Eh values of both sets of plants is shown by figure 2. For the untreated plants it amounts, by steps, to about 100 millivolts. For the treated plants the decline is much greater and, furthermore, appears to be somewhat different in type.

The low point (+330 m.v.), reached after the second night of the plants' exposure to low temperature, is of special interest. A point of this nature appeared in each one of the four tests. The only variation was the time of its appearance. In the other two tests with this variety of cabbage, which were made in April, 1941, it appeared after the fourth night of exposure rather than the second, as shown here.

In all cases this low point marked the maximal difference between the Eh values for the two sets of plants. After it was reached and passed, a substantial difference in these values continued but was lower in magnitude. Prolongation of the experiment, with additional nights of treatment, was desired but could not be effected.

Discussion

Among the findings herein reported, the most important may possibly be that of the sharp decline of the oxidation-reduction potential in response to the hardening treatment. This shift of the potential was to a remarkably lower value, meaning a sudden and large relative increase of reduction over oxidation. Doubtless this involved a change in energy relations, since the oxidation-reduction systems of biological organisms appear to be of the nature of thermodynamically reversible reactions which are characterized by electronic migrations.

Incidentally, it was noticed without exception that coincidental with the sharp drop in the potential of the treated plants there was a change in the color of the expressed tissue fluid. It was emphatically bluish-purple instead of green. This color persisted and became slightly more intense with the additional exposures of the plants to the low temperature. The leaves, in external appearance, had this same caste of color. Its general appearance in cabbage plants, undergoing hardening to cold, is an item in common experience. This is suggestive of the presence of a chromogen which may be involved in the plant's oxidation-reduction system, and which, as such, would bear particular investigation.

Summary

1. The existence of an oxidation-reduction system in the tissue fluid of the cabbage plant was experimentally demonstrated. Its electric potential, with normally grown plants, was found to be positive in character (dominance of oxidation over reduction) and to vary within the range of +450 to +475 millivolts.

2. The relationship between the pH and the Eh of the tissue fluid was found to be definite and regular. It was expressible in the mathematical form of a parabola, the equation of which was derived, and is presented.

3. Tests were made for the effect of leaf position (essentially leaf age) on the plant's oxidation-reduction potential. Although inadequate as proof, the evidence pointed to the potential being low in the young leaves; highest in the leaves at full development; and declining in magnitude as the leaves passed the stage of maturity and began to age.

4. The oxidation-reduction system of the cabbage plant gave an apparent response to the condition of exposure of the plant to low temperature (hardening). This response, consistent within the limits of the experimentation, was of the nature of a sharp drop in the potential after two to four exposures to low temperature; a slight rise in the Eh values, with added exposures;

and their continuation at a level below that of the unexposed and unhardened plants.

MICHIGAN STATE COLLEGE
EAST LANSING, MICHIGAN

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THE USE OF ION EXCHANGE MATERIALS IN STUDIES ON CORN NUTRITION[1]

C. D. Converse, Nathan Gammon, and J. D. Sayre

(with three figures)

Introduction

In 1937, a series of studies involving the use of ion-adsorbing, or ion-exchange materials was begun at the Ohio Agricultural Experiment Station in an effort to overcome some of the difficulties previously encountered in growing corn to maturity in controlled nutrition experiments. Nutrient culture techniques involving the use of these materials present many advantages over those in present use, particularly over solution cultures. Plants can be grown to full size and maturity under carefully controlled conditions. The technique permits the study of typical mineral deficiency symptoms and responses to varying amounts of the different elements. More accurate studies of the effect of the relative mineral content of the nutrient media on the mineral content of the plant parts are possible. Single elements can be varied without changing over ion concentrations. Undesirable osmotic pressures are eliminated. Changes in pH of the cultures are reduced to a minimum, and mutual precipitation of ions from solutions is avoided. The time required to care for the cultures is greatly reduced, as fresh solutions are not required at frequent intervals. The time required to set up the original cultures is somewhat greater than for solution cultures, because it is necessary to prepare different ion-exchange materials in rather large quantities in advance. Since the exchange materials must be intimately mixed with the gravel, it is not possible to change the nutrient levels of the cultures very much after they are started; thus, any mistakes in supply of the different elements cannot be corrected, nor can any addition or subtraction be made after the cultures are started. This might be a disadvantage if different conditions of nutrient supply were required for the different stages of growth of the plant.

The problem of growing plants in solution cultures has received considerable attention, and there are very extensive bibliographies of the literature on this subject. Few investigators, however, have used adsorbed ions for growing plants. Pioneer work on this subject has been done by Breazeale (**4**) and McCalla (**7**), who have studied artificial zeolites and the clay fraction from the soil and used them as a source of ions for plants. Albrecht and McCalla (**1**) give a very good account of this subject and references to previous work.

Schlenker (**8**) began work at the Ohio Agricultural Experiment Station

[1] Department of Agronomy, Ohio Agricultural Experiment Station, and the Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, cooperating.

in 1938 and carried it on at the Connecticut Agricultural Experiment Station with the same type of materials. A progress report covering the Ohio work to date was read at the meeting of the American Society of Plant Physiologists at Columbus, Ohio, in December 1939 (**5**).

Materials and methods

Materials were prepared during the winter of 1937–1938, and in the summer of 1938 corn was grown to full maturity in a large gravel tank using an iron-phosphorus-bentonite sorption complex as the sole source of iron and phosphorus. At the same time, a series of pots was set up, using zeolite with all ions adsorbed on it, supplemented with additional iron bentonite, and irrigated with distilled water. The corn in these pots grew well and, of necessity, was harvested when ear shoots were formed, as it was planted rather late. Other work of a preliminary nature, using the colloidal fraction of a Miami subsoil as a sorption complex, was begun at this time but was abandoned in favor of commercial water-softening materials, which, because of their granular nature, were much easier to prepare and handle than the bentonite and clay colloids. Although iron has been supplied successfully on the various adsorption agents, it has been used more conveniently in the form of finely divided magnetite, as suggested by EATON (**6**).

The ion-adsorbing materials used in these studies included bentonite,[2] exchange capacity, 80 mg. equivalents per 100 gm.; Miami subsoil clay colloid, 65 m.e., prepared by BRADFIELD'S method (**2, 3**) and electrodialyzed free of positive ions other than hydrogen; "Decalso,"[3] an artificial sodium zeolite, 230 m.e.; "Zeo-Karb-H,"[3] a granular base-free hydrogen exchange material made from coal, 200 m.e.; and "De-Acidite,"[3] an aniline-anion-fixing material, 120 m.e.

The iron-phosphorus-bentonite complex was prepared by dispersing the bentonite in distilled water and adding an excess of a ferric sulphate solution. After thorough mixing, the supernatant liquid was siphoned off, and more distilled water and ferric sulphate were added. This process was repeated, and the mixture of bentonite and ferric sulphate was washed with distilled water until the bentonite no longer settled out. This procedure produced an iron-bentonite. An iron-phosphorus-bentonite complex was formed by adding phosphoric acid to this mixture, and the process of saturating and washing was repeated. Finally, the excess liquid was removed by supercentrifuging, and the resulting paste was stored in glass jars.

The hydrogen clay was dispersed to about a 2 per cent. suspension and treated with the hydroxide or the carbonate of the desired element in accordance with its equivalence value as determined by electrometric titration.

The commercial materials are granular and were treated without any previous preparation. The Decalso was leached with a solution of the chloride salt of the desired cation until saturated. The Zeo-Karb-H was

[2] Furnished by the Wyodak Chemical Co., Cleveland, Ohio.

[3] Furnished by the Permutit Co., New York.

treated in a water suspension with the hydroxide or carbonate of the element desired, in accordance with its equivalence value as determined electrometrically. The De-Acidite was shaken with an excess of dilute acid of the anion desired and permitted to stand overnight. Following treatment, excess salts were washed out with distilled water on a suction filter, after which the materials were dried.

All materials were analyzed after treatment to determine the amounts of ions adsorbed. Two-gm. samples were leached with 200 ml. of normal neutral ammonium acetate, and the leachate was analyzed for the adsorbed ion by standard methods to check the quantity of ions adsorbed.

The amounts of the elements necessary in the culture media to mature a single corn plant were determined from previous analyses of both field- and solution-grown corn plants. When the quantity of the elements adsorbed on the exchange materials was known, it was simple to weigh out enough of the materials to supply the necessary elements.

At planting time, the pots and reservoirs were assembled as shown in the diagram (fig. 1). Twelve hundred gm. of no. 4 clean quartz gravel were

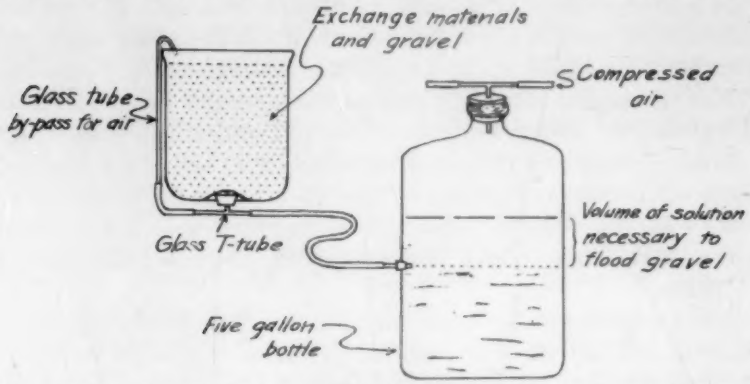

FIG. 1. Sketch of the pots and reservoirs used in cultures of ion-exchange materials.

weighed out for each pot, put into a 10-gallon barrel churn with 200 ml. of rain water and the desired quantities of exchange materials, and mixed for 5 minutes. It was found that a 3-inch baffle board fitted diagonally from top to bottom in the churn gave a thorough mix.

The pots were irrigated automatically four times daily. Seed was planted about an inch deep, and when the plants were about 4 inches high, they were thinned to one stalk at a place. Tillers were removed if they developed. From one to four stalks of corn were grown in a pot, although it was found that when four stalks were grown, the total dry weight produced per plant was reduced, apparently because of root-binding. Water which evaporated or transpired from the pots was replaced daily with rain water.

Results

This type of culture has been used satisfactorily to study growth re-

sponses and deficiency symptoms of corn to calcium, magnesium, potassium, phosphorus, nitrogen, sulphur, and boron. Typical results obtained by this method are illustrated by two experiments in which series of levels of potassium and phosphorus were used. Two plants were grown in each culture,

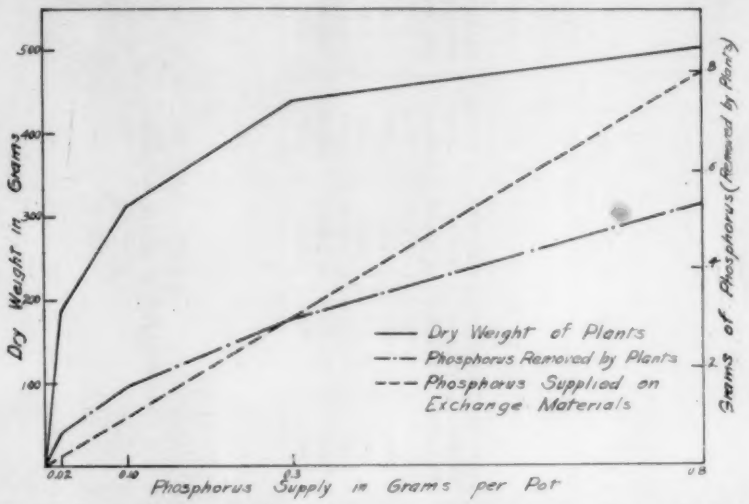
Dry Weight in Grams
Grams of Phosphorus (Removed by Plants)
Dry Weight of Plants
Phosphorus Removed by Plants
Phosphorus Supplied on Exchange Materials
Phosphorus Supply in Grams per Pot

FIG. 2. Dry weight production by the two plants in each culture and phosphorus accumulation in the four levels of supply.

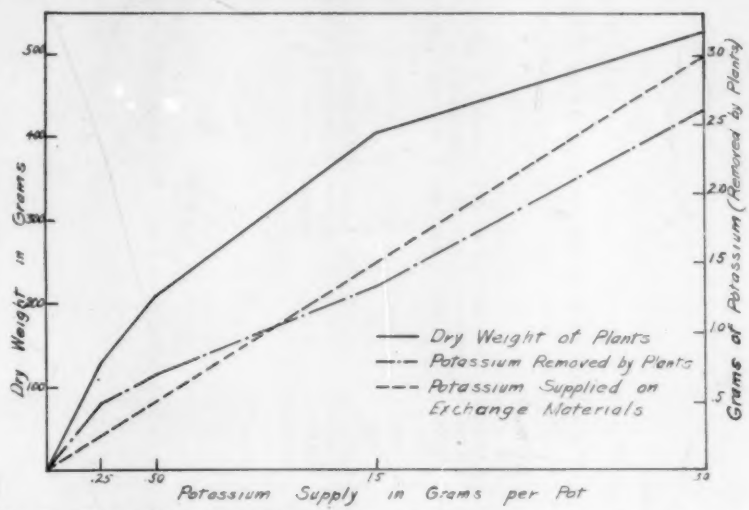
Dry Weight in Grams
Grams of Potassium (Removed by Plants)
Dry Weight of Plants
Potassium Removed by Plants
Potassium Supplied on Exchange Materials
Potassium Supply in Grams per Pot

FIG. 3. Dry weight production by the two plants in each culture and potassium accumulation in the four levels of supply.

one each of the two single crosses WF9 × 38–11 and 56 × 4–8. The series were based on four levels of supply of either potassium or phosphorus; all other nutrients were kept constant. The potassium series was replicated three times, and the phosphorus series twice. The general growth and appearance of the plants in the higher levels of nutrition were similar to those

TABLE I

RESULTS OF CHEMICAL ANALYSIS OF PLANTS GROWN AT THE FOUR LEVELS OF SUPPLY OF POTASSIUM, SUMMER 1940. EXPRESSED ON A DRY WEIGHT BASIS

ELEMENT ADDED IN THE EXCHANGE FORM	LEVEL 1			LEVEL 2			LEVEL 3			LEVEL 4		
	AMOUNT ADDED PER POT	AMOUNT REMOVED PER POT	CONCENTRATION IN PLANT	AMOUNT ADDED PER POT	AMOUNT REMOVED PER POT	CONCENTRATION IN PLANT	AMOUNT ADDED PER POT	AMOUNT REMOVED PER POT	CONCENTRATION IN PLANT	AMOUNT ADDED PER POT	AMOUNT REMOVED PER POT	CONCENTRATION IN PLANT
	gm.	*gm.*	%	*gm.*	*gm.*	%	*gm.*	*gm.*	%	*gm.*	*gm.*	%
Potassium	0.25	0.48	0.38	0.50	0.69	0.33	1.50	1.31	0.32	3.00	2.63	0.50
Phosphorus	1.00	0.236	0.184	1.00	0.205	0.097	1.00	0.342	0.084	1.00	0.413	0.078
Nitrogen*	6.00	3.01	2.35	6.00	3.31	1.57	6.00	4.16	1.02	6.00	4.62	0.87
Calcium	2.50	0.71	0.55	2.50	0.81	0.38	2.50	1.19	0.29	2.50	1.02	0.19
Average dry matter produced per pot of two plants, gm.		128			211			406			500	

* Part of the nitrogen was supplied in solution as urea. The minor elements, except iron, were supplied in dilute solutions.

TABLE II

RESULTS OF CHEMICAL ANALYSIS OF PLANTS GROWN AT THE FOUR LEVELS OF SUPPLY OF PHOSPHOROUS, SUMMER 1940. EXPRESSED ON A DRY WEIGHT BASIS

ELEMENT ADDED IN THE EXCHANGE FORM	LEVEL 1			LEVEL 2			LEVEL 3			LEVEL 4		
	AMOUNT ADDED PER POT	AMOUNT REMOVED PER POT	CONCENTRATION IN PLANT	AMOUNT ADDED PER POT	AMOUNT REMOVED PER POT	CONCENTRATION IN PLANT	AMOUNT ADDED PER POT	AMOUNT REMOVED PER POT	CONCENTRATION IN PLANT	AMOUNT ADDED PER POT	AMOUNT REMOVED PER POT	CONCENTRATION IN PLANT
	gm.	*gm.*	%	*gm.*	*gm.*	%	*gm.*	*gm.*	%	*gm.*	*gm.*	%
Phosphorus	0.02	0.074	0.039	0.10	0.162	0.052	0.30	0.296	0.067	0.80	0.525	0.104
Potassium	5.00	2.13	1.13	5.00	2.79	0.89	5.00	2.99	0.68	5.00	2.75	0.55
Nitrogen*	6.00	2.63	1.40	6.00	3.59	1.15	6.00	4.08	0.93	6.00	4.23	0.84
Calcium	2.50	0.21	0.11	2.50	0.28	0.09	2.50	0.44	0.10	2.50	0.55	0.11
Average dry matter produced per pot of two plants, gm.	188			313			439			503		

* Part of the nitrogen was supplied in solution as urea. The minor elements, except iron, were supplied in dilute solutions.

of field-grown plants, whereas typical deficiency symptoms developed in the lower levels.

Distinct differences were shown between the two hybrids used in these experiments, but the results given in this paper are average values. They are shown graphically in figures 2 and 3. The total dry weight production per culture of the above-ground portion of the plants increased progressively with increasing nutrient supply, and the amount of phosphorus and potassium accumulated by the plants increased also, but not in proportion to the amount supplied in the cultures. In fact, the results show that the plants removed more than was supplied at the lower levels, probably because of contamination of the cultures from dust or from the rain water used.

Tables I and II give a brief summary of the results of the chemical analysis of the plants grown in these two experiments and a record of the amount of each of the major elements supplied to each culture.

Discussion

None of the major elements were limiting factors except where made so, because the total amount of the element absorbed was only a small part of the amount added. A relationship between calcium and potassium is shown in table I, which records how the increased potassium absorbed by the plants reduced the percentage concentration of calcium in the plants. This is not a reciprocal relationship because the total amount of calcium was not depressed except in the last level. Relationships exist among the other elements, but it is difficult to interpret them because of the great change in plant size which occurred with increased supplies of potassium.

The results shown in table II, showing an experiment in which all elements except phosphorus were constant, are also difficult to interpret. Phosphorus increased in amount and in concentration in each level of supply. Potassium decreased in concentration as the plants became larger, but the total amount taken up increased except in the last level. Nitrogen was absorbed by the plants in greater amounts in each level but not in proportion to plant size, because the concentration decreased in each level. Calcium changed significantly in the different levels; it was very low in all.

Summary

A new technique for plant nutrition studies has been outlined, and illustrations of the type of results that may be obtained are given. This new technique permits the addition of each major element separately. Growth responses and deficiency symptoms are obtained just as easily as in solution cultures, but the responses and symptoms are more definite than in other methods of culture.

Ohio Agricultural Experiment Station
Wooster, Ohio

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ESTIMATION OF STOMATED FOLIAR SURFACE OF PINES

THEODORE T. KOZLOWSKI AND FRANCIS X. SCHUMACHER

(WITH ONE FIGURE)

Physiological investigations of forest tree species require, on occasion, the measurement of the total surface area of tree foliage. The problem of determining leaf surface areas of broad-leaved species is not as difficult as that of determining foliage surface area in coniferous species; for despite individual irregularity, leaves of broad-leaved species may be traced and their outlines planimetered. An improved technique for determining exposed leaf area of broad-leaved species is the photoelectric cell method, one modification of which is described by KRAMER (**5**). The grid-area method of NEWMAN (**7**) which is an application of the mean ordinate principle can also be used for leaves of broad-leaved species. These methods cannot be applied to the needle-like leaves of pine.

GROOM (**2**) estimated the surface of coniferous foliage by combining his measurements of numbers and lengths of leaves with those of VON HÖHNEL (**3, 4**). MINCKLER (**6**) determined leaf area in pine by taking diameter measurements with an eyepiece micrometer and making a small allowance for taper. UHL (**8**) adopted the displacement principle and suggested a relationship between surface development and volume but did not give information on taper corrections.

This paper describes an indirect method of measuring the aggregate surface area of pine needles by taking advantage of the correlation between surface area and volume of needle fascicles; for the latter may be determined directly and quickly by the measurement of displacement.

The method involves the following assumptions with respect to the cross-section of a pine needle fascicle when the component needles are in properly-oriented contact:

1. That the outline of any cross-section along the length of fascicle is circular; that departure therefrom gives rise to no greater relative error of calculated surface or volume of the fascicle than does the corresponding assumption on the calculated surface or volume of the tree bole; in short, that the consequence of departure from circular outlines is negligible.

2. That contiguous faces of needles, as viewed in cross-section, may be regarded as radii of the fascicle circle.

Observations taken on a great number of fascicle cross-sections indicate that the above assumptions are valid, unless the fascicle has undergone dehydration or has been treated for sectioning.

To calculate volumes of individual fascicles it is necessary that lengths and average diameters be determined. In order to obtain diameter measure-

ments at equidistant intervals it is necessary to bring the needles of a fascicle together in proper orientation with radial walls in contact. A satisfactory practice is one in which thread ligatures, each with a single overhand knot, are slipped on the fascicle from its sheath end. All the knots are then tightened while the ligatures are located closely together just above the fascicle sheath. In this region the needles are not twisted and the radial surfaces are contiguous. The ligatures are slipped downward toward the apex and one is placed just above or below each point of diameter measurement. Twisted needles are in this way forced into a corrected position in the fascicle. With the ligatures holding the needles together the desired diameters may be easily taken with a microcaliper.

If, then, the actively transpiring surface, that containing the stomates, of an essentially 3-needled pine, such as loblolly pine (*Pinus taeda* L.), includes the external surface area of the fascicle, together with the surface of the 6 radial faces, calculation of surface area and volume of the fascicle is easily performed. Suppose, for example, that diameter measurements, d_1, d_2, d_3, d_4, and d_5 are taken at equidistant intervals along the fascicle from base to tip. Since d_5 represents the tip diameter its value, obviously, is zero. Consequently if the length of the fascicle be denoted by l, then the outer surface, o, of the fascicle may be represented

$$o = (\pi(l)/5)\,[d_1 + d_2 + d_3 + d_4]$$

while the inner surface, i, may be expressed

$$i = (3(l)/5)\,[d_1 + d_2 + d_3 + d_4]$$

Hence the entire transpiring surface, y_L, of a loblolly pine fascicle is the sum of these components, that is

$$y_L = o + i = (l/5)\,(d_1 + d_2, + d_3, + d_4)\,(\pi + 3) \quad \ldots\ldots\ldots\ldots (1)$$

The volume, x, of the same fascicle may be derived as readily. Since volume is the product of length, l, and the average cross-sectional area, it follows that

$$x = (l/5)\,(d_1^2 + d_2^2 + d_3^2 + d_4^2)\,(\pi/4) \quad \ldots\ldots\ldots\ldots\ldots\ldots (2)$$

By contrast, the expression for the transpiring surface of a particular 5-needled pine, such as eastern white pine, (*Piuus strobus* L.), differs from equation (1) in that stomates occur only on the radial faces of the needles of a fascicle. In this discussion it is assumed that transpiration from non-stomated surfaces is negligible and these surfaces are therefore neglected. In this case, then, o is zero, and the entire transpiring surface, y_W, may be represented

$$y_W = l\,(d_1 + d_2 + d_3 + d_4) \quad \ldots\ldots\ldots\ldots\ldots\ldots (1a)$$

The expression of fascicle volume of a 5-needled pine is, of course, identical with that of equation (2).

The transpiring surface and the volume of each fascicle of a random sample of 25 seedling loblolly pine needle fascicles were calculated according to equations (1) and (2), respectively, after taking the fascicle diameter measurements d_1, d_2, d_3, and d_4, in hundredth millimeters, and fascicle

length, l, in millimeters according to the above observational program. Upon plotting surface, y_L, on volume, x_L, of the individual fascicles in a system of rectangular coordinates, as in fig. 1 (upper graph), it was surprising to

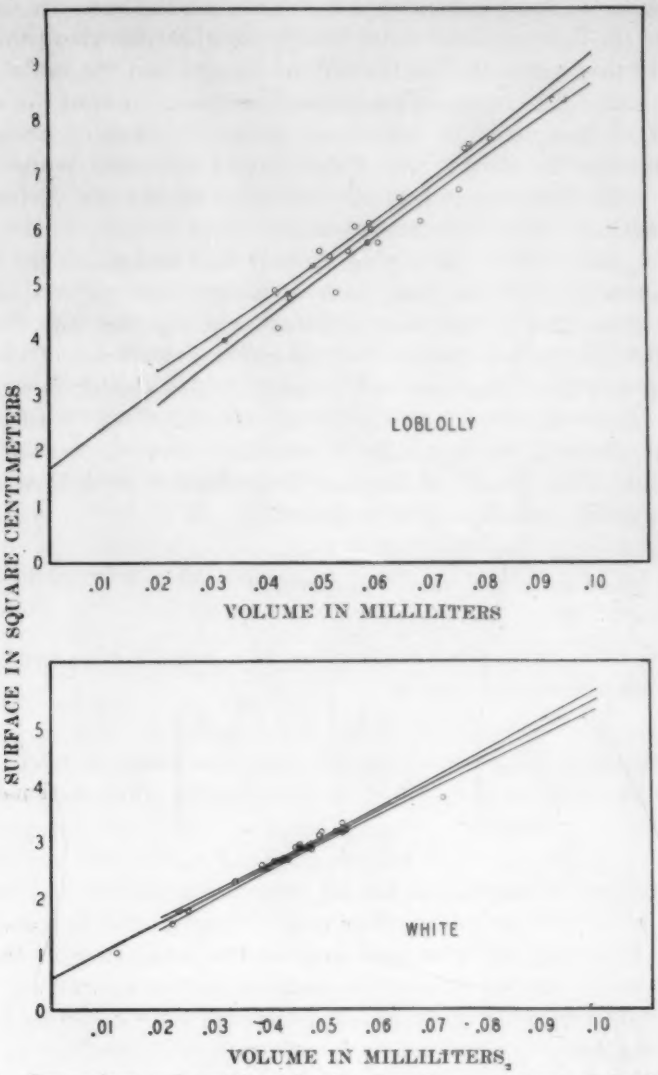

FIG. 1. Regressions and confidence bands of surface on volume of fascicles of loblolly pine and white pine. Based on data from 25 fascicles selected at random from each species.

find that the relation between the variables is not of a complex nature as had been anticipated; for it may, evidently, be adequately expressed by a straight line fitted to the plotted points.

Corresponding measurements were taken on a random sample of 25 fascicles of seedling eastern white pine needles using equations (1a) and

(2), respectively, in calculating surface and volume. Again, the discrepancies of the plotted points from the straight line fitted thereto (fig. 1, lower graph) substantiate the adequacy of the straight line.

For determining aggregate volumes of large groups of fascicles the immersion method was employed. Several techniques were investigated and it was found that methods in which overflow water was weighed are not very suitable in this work. In immersing an aggregate of fascicles in water air bubbles are often trapped in the network as it is placed in the vessel. A suitable method is one in which graduated cylinders are used and not completely filled before immersion. Trapped air bubbles may be released by pressing a bundle to the bottom of the cylinder with a glass rod.

The outline of the scheme of calculating the entire transpiring surface of any larger group of fascicles now becomes apparent. If, for instance, a 3-year-old loblolly pine has an aggregate of 1,000 fascicles, and the volume of the aggregate, as determined by immersion and the measurement of displacement, is 55.0 ml., one may enter the upper graph of figure 1 with the average volume to the fascicle (in this case, 0.055 ml.) and read therefrom the corresponding average surface (in this case, about 5.6 sq. cm.). Thus the entire transpiring surface of the 1,000 fascicles is about 5,600 sq. cm.

The experiment on transpiration rates which suggested the present problem required a high degree of precision of the calculated transpiring surface, as the transpiration rate per *unit* of transpiring surface of loblolly pine was to be compared with the corresponding rate of white pine under laboratory conditions. To this end a straight line of the form

$$y = a + b\ (x - \bar{x})$$

was fitted to the plotted points of figure 1, according to the method of least squares of FISHER (1) separately for each species. In this expression, a is the arithmetic mean in square centimeters of the 25 surface calculations of the sample for one of the species, and $\bar{x}$ is the mean in cubic centimeters of the corresponding volume calculations; while b is the computed rate of change of surface to the unit change of volume.

Upon applying the method to the sample of 25 loblolly pine fascicles as a case illustration, the expression of surface in terms of volume was found to be

$$y_L = 6.078 + 70.880\ (x - 0.0621) \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots (3)$$

and the square of the standard deviation of the discrepancies between observed y_L and corresponding y_L is

$$s_L^2 = 0.07785$$

in (sq. cm.)^2. The estimate of the standard error of any calculated Y_L is the square root of

$$0.07785\ [1/25 + (x - 0.0621)^2/0.007452] \quad \ldots\ldots\ldots\ldots\ldots\ldots (4)$$

The straight line in the upper graph of figure 1 is the representation of equation (3) for loblolly pine. The band which this line bisects throughout its length is known as the 95 per cent. confidence band. Its vertical distance (in positive and negative direction) from the bisecting line is twice the

standard error of the calculated Y_L as given by equation (4). It thus delimits the range which contains the true surface area, corresponding to given fascicle volume, with a probability of 0.95.

If equation (3) is now applied to the 1,000 fascicles, the mean volume of which, as used in the illustration above, is 0.0550 ml., the calculated average transpiring surface is the following:

$$Y_L = 6.078 + 70.880\,(0.0550 - 0.0621)$$
$$= 5.575 \text{ sq. cm.}$$

The estimate of the standard error of this value is found upon inserting 0.0550 for x in equation (4); whence the standard error of 5.575 is estimated to be the square root of 0.003641, or 0.0603 in square centimeters; and the entire transpiring surface area of the thousand fascicles is

$$1{,}000\ (5.575 \pm 0.0603) = 5{,}575 \pm 60.3$$

in sq. cm. If the standard error be doubled it expresses the 95 per cent. confidence interval. Hence, with probability of 0.95, the true aggregate surface of the thousand fascicles lies within the range

$$5{,}575 \pm 121$$

sq. cm. or within 2.2 per cent. of the calculated value. The estimate thus possesses quite a satisfactory precision, derived as it is, from careful measurements taken on only 25 fascicles. It is, of course, subject to unknown systematic error if the assumptions upon which the calculations of surface and volume of the 25 fascicles had been based, are not valid.

Summary

1. The surface area of a population of pine needle fascicles may be readily calculated through its correlation with volume, a value more easily determined by displacement.

2. A linear relationship of the form

$$y = a + b\,(x - \bar{x})$$

was found for needle fascicles of loblolly pine and eastern white pine. In the above expression, a is the arithmetic mean in square centimeters of all surface calculations of the sample for one of the species, and $\bar{x}$ is the mean in milliliters of the corresponding volume calculations; while b is the computed rate of change of surface to the unit change of volume.

3. The surface area of a population of fascicles may be readily determined by substituting in the equation above and determining the surface area of the average needle fascicle. By multiplying this value by the number of fascicles in the population the total surface area is easily obtained.

4. In establishing the graphic relationship between surface and volume of needle fascicles from a sample a microcaliper may be used to obtain diameter measurements along fascicles.

5. Thread ligatures are suggested as aids in obtaining diameter measurements.

This study was carried out at the School of Forestry, Duke University, and the writers are indebted to Dr. PAUL J. KRAMER of that institution for critical aid in the preparation of the present contribution.

DUKE UNIVERSITY
DURHAM, NORTH CAROLINA

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BRIEF PAPERS

A TECHNIQUE FOR TREATING SMALL SEEDLINGS WITH COLCHICINE

ROSS C. THOMPSON

(WITH TWO FIGURES)

Numerous techniques have been employed by various workers in attempts to induce polyploidy in plants by the use of colchicine. No one procedure appears to be the best for all kinds of material, because the technique must be varied to meet the requirements of the particular material and conditions.

There is fairly general agreement that the colchicine treatment of germinating seeds and very small seedlings is more likely to result in polyploids than the treatment of growing points of older plant organs. The writer has found this to be especially true in the treatment of several species of Lactuca. Several methods of applying colchicine to the growing points of large plants, as in lanolin paste or water-glycerine solution by immersion or with saturated cotton, have given poor results. These methods have all resulted in deformed growing points with varying amounts of polyploid and diploid tissues. In nearly every case, however, where lateral buds were treated, the tissues returned largely to the diploid form, and seeds containing only embryos having the diploid number of chromosomes resulted. In contrast to the high percentage of failures resulting from the treatment of growing points on older plants, the number of polyploids obtained from the treatment of germinating seeds and small seedlings has been quite high. However, neither the germination of Lactuca seed in weak concentrations of colchicine nor the soaking of seed in colchicine solutions previous to germination has proved entirely satisfactory. While a fairly high percentage of tetraploids has been obtained from both of these methods they have resulted in extreme retardation of radicle and root development with a consequent loss of many of the seedlings before they developed sufficient root systems to continue growth.

A method of treatment has been worked out whereby the growing apex of the seedling can be treated without subjecting the radicle and rootlets to the deleterious effect of the chemical. The procedure permits the treatment of a large number of small seedlings with a limited amount of labor. For successful application it is necessary that the seedlings have relatively long hypocotyls. The length of the hypocotyl in many seedling plants can be controlled to some extent by conditions just following the breaking of the seed coat in the process of germination, and before treating with colchicine.

In Lactuca seedlings the length of the hypocotyl has been greatly increased over normal by placing the germinating seeds in darkness on a moist surface at a temperature favorable for rapid cell activity (25°–30° C.) at

the time the radicles appear through the seed coats. In the absence of light the hypocotyls become much elongated and etiolated. When the elongation has reached its maximum, or about 20 mm., the seedlings are ready to be given the colchicine treatment. The conditions required for forcing hypocotyl elongation and etiolation may have to be altered somewhat for different kinds of material, and for some species the procedure is not at all practicable; however, it can be used to good advantage with seedlings of a great many species.

Materials and methods

A Petri dish and a disc of paper about one centimeter larger in diameter than the dish are required for each treatment. The paper can be prepared easily by folding a filter paper, in the manner indicated in figure 1, and

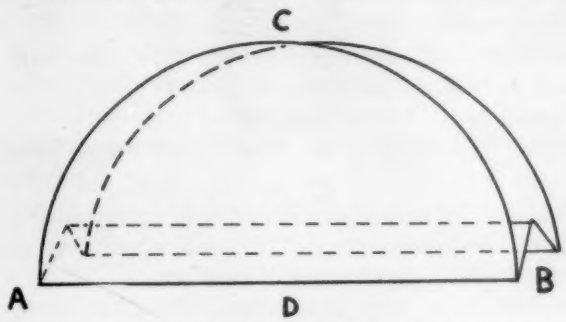

FIG. 1. A filter paper properly folded for cutting the disc to fit the bottom of a Petri dish. The distance A–B should be the same as the diameter of the bottom of the dish. The distance from C to D should be slightly less than one-half of A–B.

from this cutting a semi-circular piece about equal to one-half of the bottom of the dish. The paper is then placed in the dish as shown in figure 2 and sealed in place with melted paraffin. The dish may be slightly warmed to

FIG. 2. A Petri dish equipped for the treatment of small seedlings with colchicine solution.

prevent too rapid cooling of the wax. With the paper held in place a small amount of hot paraffin is poured into the dish while it is held in an inclined position and so that the paraffin is made to flow around the margin of the paper, sealing it to the bottom of the dish. After a few rotations the paraffin will be cooled sufficiently to hold the paper in place. A little more warm paraffin may be applied at the ends of the ridge in the paper so as to seal it to the dish at both ends. The apex of the ridge should be greased with a little vaseline or some other non-injurious grease or oil to prevent the solutions, to be placed in the dish, from creeping over the ridge and becoming mixed; or the whole disc of paper may be treated with hot paraffin. The dish is now ready for use. Enough colchicine of the desired concentration is placed on one side of the ridge to come within about $\frac{1}{8}$ inch of the top of the ridge. The height of the ridge formed by the paper may be varied so as to provide a proper depth of the colchicine solution to cover the cotyledons and epicotyls of the seedlings being treated. The other half of the dish is filled with water. It is advisable in some cases to use a layer of damp cotton batting in place of the water for keeping the roots moist.

The elongated hypocotyls of the seedlings are placed across the ridge so that the roots are in the water or on the wet cotton, and the epicotyls in the colchicine solution. In some cases it has been found advisable to apply a little vaseline to the hypocotyls at the point where they cross the ridge. This can be done with a small camel's hair brush. If the hypocotyls are long it is not difficult to keep both ends of the plant submerged. If the hypocotyls are short it may be necessary to weight the ends down lightly to keep them submerged. The dishes may then be covered and left in a desirable light and temperature for the duration of the treatment.

The number of seedlings that may be treated in a dish at one time will depend on the size of the Petri dish used and the size of the seedlings.

After the seedlings have been exposed to the treatment for the desired time, usually 4 to 8 hours, they are removed and washed thoroughly in water, dried slightly on absorbent paper, and transplanted to soil where they are permitted to grow until the effect of the treatment can be determined. Because of the long hypocotyls of seedlings thus treated it is necessary that they be set deeper in the soil than is normal. Tetraploid stems on diploid roots obtained by the procedure outlined have been far more satisfactory than the types of tissues obtained by other methods so far tried.

UNITED STATES DEPARTMENT OF AGRICULTURE
BUREAU OF PLANT INDUSTRY
WASHINGTON, D. C.

A SIMPLIFIED INTEGRATING LIGHT RECORDER FOR FIELD USE[1]

V. G. Sprague and E. M. Williams

(with two figures)

In a previous paper (**1**) an inexpensive integrating light recorder was described which operated with current supplied from a transformer and rectifier connected to an ordinary alternating current line. This paper describes a similar unit in which batteries are used to supply current. In both instruments the minute current from a phototube charges a condenser producing a potential difference which gradually increases. When the potential across the condenser reaches the breakdown voltage of a cold cathode relay tube in parallel with the condenser plates, the gas in the tube ionizes discharging the condenser and at the same time discharging a larger condenser through a sensitive counter. A direct current supply is required to supply the phototube and provide the charge on the condenser which actuates the counter.

In order to make the integrating light recorder portable for use under field conditions, the original circuit was slightly modified to use dry batteries as a source of power. The circuit (fig. 1) is simplified by this change and the action of the counter is more positive. Since the current used in operation is very small (less than 10^{-6} amperes) very small batteries are adaptable and the useful life is approximately the shelf life of the batteries. The voltage required for operation of the phototube is reduced slightly (to 90 volts for convenience in tapping when 45-volt batteries are used), while the voltage required for operation of the counter is reduced considerably, from 280 volts in the alternating current instrument to 135 volts when batteries are used. The 250,000-ohm resistor across the condenser (C_2) used to actuate the counter in the alternating current instrument is not required since the batteries can be tapped directly for the required voltage. It has been found that an 0.5-mfd. high grade paper condenser is approximately the correct capacity for condenser C_1 and that a 2-mfd. paper condenser is usually satisfactory for condenser C_2. A 250,000-ohm resistor is placed in series with the +135-volt terminal of the battery and condenser C_2 to prevent continuous ionization of the gap between the control anode P_2 and the cathode K of the relay tube.

A receiver, similar in design to that described by Wallace (**2**), has been used for two years and has been found to be quite satisfactory. A spherical globe (fig. 2) is countersunk into a wooden block through which a square hole has been cut immediately below the globe, approximately the same size

[1] Contribution no. 41, of the U. S. Regional Pasture Research Laboratory, Division of Forage Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, in cooperation with the northeastern states and the Electrical Engineering Department of The Pennsylvania State College, State College, Pennsylvania.

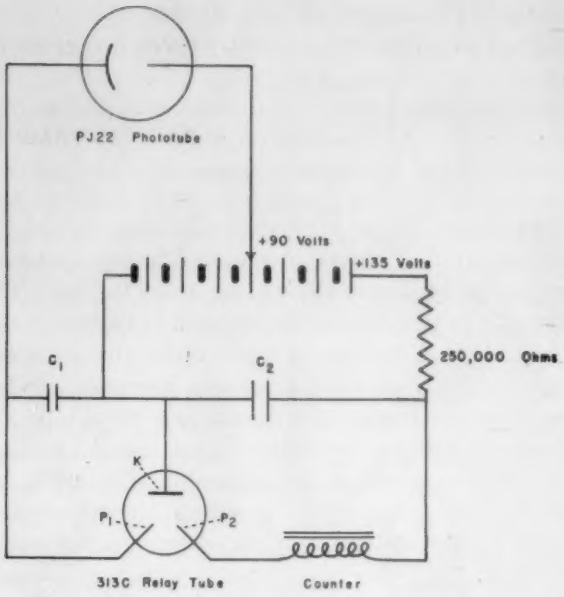

FIG. 1. Wiring diagram of battery-operated integrating light recorder.

as the opening in bottom of the globe. The phototube is mounted in a light-tight box which is fastened to the under side of the wooden base. The receiving surface of the phototube faces the globe. Inasmuch as the maximum incident intensity on the phototube should not exceed 500 foot candles, it is necessary to reduce the received intensity by placing a piece of frosted opal glass at the base of the globe and at the top of the square hole. This acts as a receiver for the light transmitted by the globe and in turn as a transmitter of light to the phototube directly beneath it. With this arrangement

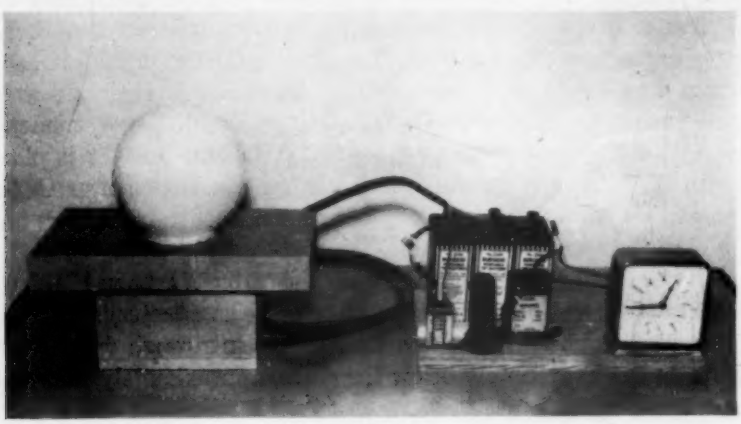

FIG. 2. Battery operated integrating light recorder and the opal glass spherical receiver.

the light reaching the phototube rarely exceeds 450 foot candles. A lead-covered cable provides a satisfactory weatherproof lead-in from the receiver to the recorder.

In calibrating the instrument a clear day without clouds is most satisfactory. A heavy paper cylinder at least four feet long and just the diameter of the globe is used. One end of the cylinder is placed over the globe and the other is pointed directly at the sun so that only parallel light rays strike the receiver. The base and other portions of the globe are covered with dark cloth to prevent other light from striking the globe. With a stop watch, the number of discharges per minute is determined and immediately thereafter the tube is removed from the globe and the intensity of the light at the base of the paper cylinder is read with a standardized foot-candle meter. Several readings should be taken which may then be used in calculating the calibration factor for the globe, phototube, and instrument. Since phototubes vary slightly in sensitivity, it is necessary to obtain a new calibration factor if the phototube is changed. Although phototubes have nearly constant sensitivity, it is probably desirable to recalibrate the instrument every six months and to remove any dirt or dust which may have accumulated on the globe, on the frosted opal glass plate, or on the phototube. If the recorder is used under field conditions, it is advisable to place everything but the receiver under cover. It should be subjected, however, to free circulation of air, since excessive ambient temperatures result in decreased battery life. Such stations as those used by the Weather Bureau to determine maximum and minimum temperatures and humidities are satisfactory. Inasmuch as this recorder develops no heat during operation, it may be thoroughly enclosed to keep dust and blowing rain from reaching the instrument. The total cost of the parts for this instrument was approximately $12.00.

It is probable that an alternating current instrument is preferable where continuous records for a number of years are desired and where the recorder can be placed in the laboratory; but for field operation, or wherever a portable recorder is required, the battery operated set is especially adaptable.

U. S. Regional Pasture Research Laboratory
and
The Pennsylvania State College

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A LIGHT-TIGHT BOX FOR MAKING SHADOWGRAPHS OF AVENA SEEDLINGS FOR GROWTH HORMONE DETERMINATIONS

R. E. Bennett, S. A. Gordon, and S. G. Wildman

(WITH ONE FIGURE)

The authors have made and are using a simple light-tight box for making shadowgraphs of Avena, quickly and easily, without leaving the constant-temperature-constant-humidity culture room. After visiting other laboratories, and talking to other workers it is our impression that usually the rows of seedlings are taken out of the culture room to an adjacent dark room for making the exposures. To shadowgraph 38 rows requires opening the door 76 times, allowing large volumes of dry cool air to enter the culture chamber. Tests with a hygro-thermograph showed that the humidity varied more than 20 per cent. during the interval that shadowgraphs were being made, whereas at other times it remained nearly constant. A further disadvantage of carrying the seedlings to another room is the danger that the coleoptiles may be bent or disarranged while opening and closing a heavy door such as is used for a culture room.

By experimentation it was determined that the distance between the light and the seedlings could be decreased to less than six feet without introducing any appreciable error from parallax. Sharply defined shadows were obtained by using a clear (non-frosted) bulb. Even more sharply defined shadows were obtained with a six-volt automobile tail-light bulb for illumination.

The essential features of the box which was designed and built are shown in the accompanying figure. Since the box is used inside the culture room under conditions of high humidity, it is suggested that waterproof plywood be used in construction to minimize warping. All joints should be glued with a resin glue such as "Cascamite" or "Weldwood." White light is confined to the interior of the box by the use of baffles on the door, instead of felt, because a slight warping or a poor fit would allow light to leak around felt. A ruby bulb is placed on the outside and is wired in series with the clear (non-frosted) 40-watt bulb which is inside the box. The ruby bulb serves as a pilot light and also reduces the voltage on the white bulb so that exposures with "Kodabrom" contrast bromide paper are about four seconds. A switch is placed on the cord rather than on the box, to minimize vibration during exposure. A small ruby bulb (not shown in the figure) is placed inside the box to facilitate handling the seedlings and the photographic paper. This bulb is connected directly to the line so that it remains burning all of the time.

The use of this box in the culture room has considerably speeded and facilitated the shadowgraphing of Avena seedlings. It is small enough to cause no inconvenience in the culture room. No white light escapes from the box. In this laboratory two persons work together and use as many as

38 rows of seedlings for one experiment. While one person is finishing the placing of agar blocks the other can begin shadowgraphing, with confidence

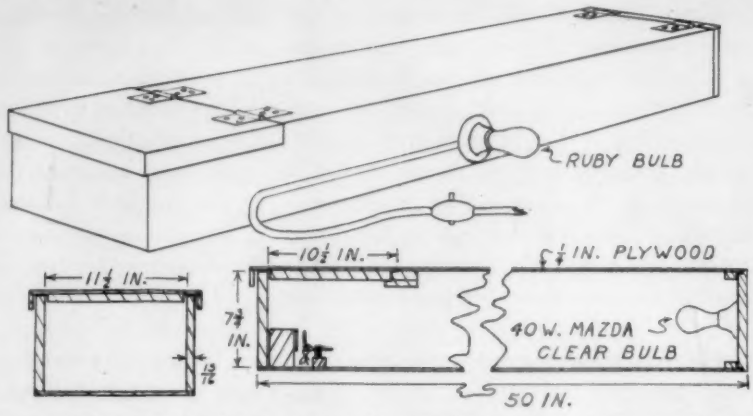

FIG. 1. Above, a light-tight box for making shadowgraphs of Avena seedlings; below, left, cross section of the box showing construction of the door; right, median longitudinal section of the box showing placement of the paper holder, seedling holder, and the white light.

that the last part of the experiment will not be altered by humidity or temperature changes.

UNIVERSITY OF MICHIGAN
ANN ARBOR, MICHIGAN

NOTES

Meeting Canceled.—At the request of the Office of Defense Transportation the meetings which were to have been held in New York late in December, 1942, were postponed or canceled in order to relieve the congestion of army holiday travel. In most respects we can go along about as well without the meetings; but it does make it more difficult to have intelligent consideration of the business matters which should have received attention at the annual meeting. It is hoped that the Executive Committee will not consider any matter settled until the reasons for proposed actions can be thoroughly understood, and appropriately digested. The main committee reports will be presented in this number of PLANT PHYSIOLOGY.

Life Membership Award.—The CHARLES REID BARNES life membership committee reported the selection of Dr. OLENUS LEE SPONSLER, Professor of Botany at the University of California at Los Angeles, to receive the nineteenth award of this honor. The award is made in recognition of Dr. SPONSLER's contributions to the knowledge of molecular structure of biological materials as revealed by x-ray methods. Dr. SPONSLER is a native Ohioan, with degrees from Michigan, Nebraska, and Stanford. He has been connected with the University of California since 1922. The Society is greatly honored by the addition of Dr. SPONSLER to its roll of CHARLES REID BARNES life members.

Hales Award.—The eighth award of the STEPHEN HALES prize has been made to Dr. CORNELIS BERNARDUS VAN NIEL, Professor of Microbiology at the Hopkins Marine Station, Stanford University, in consideration of his brilliant work on the biochemistry of lower organisms, and particularly his contributions to an understanding of photosynthesis among these organisms. Dr. VAN NIEL was born at Haarlem, Holland, November 4, 1897. He was trained at the Technical University at Delft, where he obtained his degrees, and where he served as conservator of the microbiological laboratory from 1924 to 1928. In the latter year he was attached to the Hopkins Station, where he has worked for 14 years.

His best known work deals with bacterial photosynthesis. When he first began to study the purple and green sulphur bacteria (*ca.* 1926) knowledge of the nutrition and metabolism of these organisms was fragmentary and contradictory. Several theories had been advanced, some believing that photosynthesis was, in these forms, not much different than in green plants; others believed them to be chemosynthetic; still others thought the metabolism was a mixed chemosynthetic photosynthetic process. The difficulty was to reconcile the following observations:

1. Light and oxidizable sulphur compounds are required.
2. No oxygen is liberated.

3. The sulphur compounds are oxidized generally to sulphates in the absence of oxygen.
4. Carbon dioxide is consumed.

Dr. VAN NIEL (1930–1931) clarified the problem by a combined experimental and theoretical approach. He isolated (for the first time) pure cultures of purple and green sulphur bacteria, studied the influences of light, oxygen, oxidizable sulphur compounds, carbon dioxide, etc., on their growth, and established the quantitative relations among the substances consumed (CO_2, H_2S) and produced (SO_4, cell material) in the light of metabolism. The experimental results were interpreted in terms of a new type of photosynthesis which, when H_2S is the oxidizable substrate and sulphur the oxidation product, is expressed by the equation:

$$CO_2 + 2H_2S \rightarrow (HCHO) + H_2O + 2S$$

generalized, thus: $CO_2 + 2H_2A \rightarrow (HCHO) + H_2O + 2A$,
where H_2A is any oxidizable substrate, organic or inorganic.

These bacterial photosyntheses are sharply distinguished from green plant photosynthesis by their dependence upon the presence of an oxidizable substrate other than water, and by the absence of any oxygen production.

VAN NIEL's generalized theory of photosynthesis has had an important influence upon subsequent experimental studies of green plant photosynthesis; witness the work of GAFFRON on photosynthetic hydrogen utilization by Scenedesmus, and RUBEN's demonstration by the use of isotopes, that the oxygen of photosynthesis is derived from water, not from carbon dioxide.

In addition to these studies of photosynthesis, VAN NIEL has contributed important papers on the morphology, nutrition, taxonomy and biochemistry of propionic acid bacteria; and his work on the fermentation of glycerol provided an essential link in the chain of events which led to the discovery of the rôle of carbon dioxide in the formation of succinic acid and other products by WOOD and WERKMAN in 1936. He has also made important contributions to bacterial taxonomy in general, in collaboration with KLUYVER and STAVIER.

Other subjects which have claimed his attention are methane fermentation, fermentative assimilation, diacetyl and butter aroma, and a new genus of yeast-like organisms, Sporobolomyes.

Not the least of his accomplishments is his ability as a teacher. An actor at heart, he has the rare gift of being at the same time precise, logical, and inspirational. These and other qualities make him the idol of his students, the wonder and delight of his friends.

The committee on the award, made up of the last three recipients of the award, have made a splendid selection, one which merits universal approval. It accords with the spirit of the Society, and with the spirit of those who, in 1927 and later years, by their gifts, laid the foundations for this biennial award. We can all be justly proud that the honor has been bestowed on one so well deserving it.

Editorial Report.—In reporting the activities of the editorial committee for 1942, attention was directed to several matters of general interest. The volume for 1942 showed considerable reduction in size from that of 1941, a total of 717 pages as compared to 857 in 1941. The reduction came about without sacrifice, because the demands for space were smaller in 1942 than in 1941. For instance, the 66 papers published in 1941, averaged 13 pages each; whereas the 70 papers published in 1942 averaged only 10 pages each. The decrease in size of volume 17 is, therefore, an expression of the more concentrated and less verbose type of paper which has been coming into favor among scientists generally. This is an encouraging trend, and, if it is maintained, should cushion the impact of war upon our finances. Authors should keep in mind at all times that care in the preparation of manuscripts will dispense with unnecessary repetitions, will not present or stress the obvious and well-known facts, and will reduce summarizing of past literature to the minimum requirements. The editors appreciate the progress investigators are making in the direction of succinct reports, but desire to retain our practice of giving space to lengthy papers when more space is needed.

George Peter Hoffman.—On April 1, 1941, GEORGE PETER HOFFMAN, who was connected with the U.S.D.A. horticultural field station at Meridian, Mississippi, died of a heart ailment. He was born in Columbia, South Carolina, February 23, 1890. His advanced education was obtained at Clemson College, where he was graduated in 1915; and at Cornell University (M.S., 1925), and Michigan State College, 1932. He was an assistant in horticulture at Clemson in 1916, went into extension work with them for about six years, first in general extension work and then as extension horticulturist until 1923. He then entered the Department of Horticulture as associate professor, a position which he filled for three years. In 1926 he went with Penney Farms, the J. C. Penney-Gwinn Corporation, which he served for six years as manager of its program. In 1932 he was appointed to a position in the Department of Agriculture in nut investigations, and was soon stationed at Meridian, where his work was mainly concerned with pecans, grapes, sweet potatoes, and vegetables.

He is survived by his wife, who lives at Fountain Inn, South Carolina, and a son who is in attendance at Clemson College.

Fellowship.—Sigma Delta Epsilon continues its award of a $1000 fellowship. The second award is to be made for 1943–1944, and applications and references should be submitted before March 1, 1943. Women with the equivalent of the master's degree, with research in mathematical, physical, or biological sciences, who need aid to complete their work, are eligible. They must give evidence of high ability and promise in research. Those receiving the award are supposed to devote the major time to research on some approved subject, and not to do work for remuneration outside the fellowship, unless such program has been specifically authorized and approved.

Application blanks may be obtained from Dr. ELOISE GERRY, care of the U. S. Forest Products Laboratory, Madison, Wisconsin. Interested persons are invited to write, and to mark correspondence "Personal."

Sigma Delta Epsilon may well be proud of its determination to carry on this valuable work during these desperate times.

Selective Service.—While the rules for selective service and selective deferment are so much subject to change that statements do not remain accurate for very long, the following notes are culled from a statement on occupational deferment issued under date of September 30, 1942.

The War Manpower Commission has certified that educational services are essential to the support of the war effort. Under such services the Commission specifies public and private industrial vocational training; elementary, secondary, and preparatory schools; junior colleges; colleges, universities, and professional schools; educational and scientific research agencies; and the production of technical and vocational training films.

A list of critical occupations is given in occupational bulletin 23, and among others the following are of interest to our membership: Agricultural sciences, bacteriology, biology, chemistry, and physiology. In general, occupations requiring more than 6 months of technical training are to receive consideration at the hands of all draft boards. Full time instructors in biology in elementary schools, and presidents, deans, and registrars in junior colleges, professors and instructors engaged in full-time instruction in the subjects mentioned, are considered as engaged in work critically related to the war effort.

This information is given because our members may not be aware of the privileged status of their work as full time instructors in critical occupations.

Carnivorous Plants.—One of the most interesting and romantic chapters in plant physiology is that which deals with the carnivorous plants. Those who have been privileged to read the account in KERNER and OLIVER'S *Natural History of Plants* which deals with these interesting creatures, will turn to a new account of them with expectant enthusiasm. For more than a decade Dr. FRANCIS E. LLOYD has been studying these plants intensively, and has now brought his observations into book form. It is a handsome monograph entitled *The Carnivorous Plants*. It is published by the Chronica Bontanica Company, of Waltham, Massachusetts.

The introductory chapter gives a general account of these curious plants, their geographic distribution, the types of traps and pitfalls that have been developed, and the difficulties of accounting for them on any evolutionary hypothesis. The reviewer has always wished to hear some survival-of-the-fittest philosopher set himself to the task presented by these complicated trapping devices.

The succeeding chapters take up genera or species for detailed consideration, and the chapters bear the following titles: Heliomphora; Sarracenia; *Darlingtonia californica;* Nepenthes; *Cephalotus follicularis;* Genlisea; By-

blis; *Drosophyllum lusitanicum;* Pinguicula; Drosera; carnivorous fungi; Dionaea; Aldrovanda; Utricularia, Biovularia and Polypompholyx; and the Utricularia trap.

The illustrations occupy 38 plates at the close of the text, and these drawings help to make the text descriptions clear. It is a delightfully written account, and summarizes as well as can be done at present, the construction and operation of the trapping devices described. One can well imagine that the author of the work found the study most fascinating, and the work as a whole is a monument to Prof. LLOYD'S industry and scholarship.

The book is volume 9 in a series edited by FRANS VERDOORN. The price of the book is $6.00 per copy. Orders may be sent to Waltham, Mass., The Chronica Botanica Co., or New York City, G. E. Stechert and Co., the publishers.

Autonomic Regulations.—A book of interest to premedical and medical students, and of physiologists by Dr. ERNST GELLHORN, of the College of Medicine of the University of Illinois, deals with autonomic regulations in the animal body. The general introduction presents the anatomical and physiological foundations of the subject.

The second section deals with adjustment reactions involving primarily the respiratory and circulatory systems, such as adjustment reactions to carbon dioxide, anoxia, asphyxia, hemorrhage, hypoglycemia, and regulation of cerebral circulation.

Part III discusses autonomic-endocrine integration; part IV, autonomic somatic integration; and part V with applications, and the physiological and clinical aspects of autonomic regulations.

Although less valuable to plant physiologists than to animal and human physiologists, it should stimulate thinking in comparative terms of plant and animal reactions. It is a book of 373 pages, and is published by Interscience Publishers, Inc., 215 Fourth Ave., New York. The price is $5.50 per copy.

Immunology.—A book on fundamentals of immunology, written by Dr. WILLIAM C. BOYD, of the Boston University School of Medicine, surveys the information in this field. It contains eleven chapters, as follows: Immunity and immunology; antibodies and antibody specificity; antigens; cell antigens; blood groups; antibody-antigen reactions; complement and complement fixation; anaphylaxis and allergy; allergy and immunity, bacteria, viruses, parasites; practical use of artificial immunity; and laboratory and clinical techniques.

Certain phases of this work should be of particular interest to plant pathologists, and virus investigators. It is intended primarily for the student of medicine and animal biology, but again has value in comparative physiology and pathology of animal and plant life.

It contains 446 pages, and is quoted at $5.50 per copy. The publishers are Interscience Publishers, Inc., New York. Biological libraries should be expected to make works of this type available to all biological investigators.